Cellular & Molecular Biology
SQBS 1143

Chapter 5:
Eukaryotic Genetics

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Definition of Eukaryotes

- Most of the basic principles of molecular biology were discovered using bacteria. This is because bacteria are relatively simple and easy to use in experiments.

- By definition, possession of a nucleus makes you an eukaryote. The **nucleus** is a separate compartment of the cell and is surrounded by a membrane, the **nuclear membrane**. The nucleus is where higher organisms keep the chromosomes.

- Lower organisms such as bacteria are known as prokaryotes and typically have **two or three thousand genes** carried on a single **circular chromosome**.

- In contrast, eukaryotes have **many thousand genes** carried on several **linear chromosomes**.

- Humans and fruit flies are both estimated to have between **50,000 and 100,000** genes. The difference between you, dear students, and some benighted insect is not so much the number of your genes, but their organization.

Cell Structure in Eukaryotes

- Eukaryotic cells are much bigger than bacterial cells and are divided into **separate compartments by membranes**.

- In addition to the nucleus, eukaryotic cells have several other compartments surrounded by membranes. These are known as **"membrane bound organelles"** since they are like the organs of animals and plants but on a miniature scale.

Mitochondria are Used for Respiration

- The most common organelles are the **mitochondria** (singular, *mitochondrion*). Almost all eukaryotic cells have mitochondria, which are responsible for **generating energy** by the **oxidation of food molecules**.

- This process is known as **respiration**. You might think you are respiring when your lungs suck some air, but you are only breathing.

- To qualify officially as respiration, the **oxygen from the air must reach the mitochondria** in your cells and be used **to burn the food molecules** as fuel. Otherwise, the air does you no good.

Chloroplasts Trap Energy from the Sun

- **Chloroplasts** are organelles found only in plants. They carry out, **photosynthesis** a process of trapping the energy in sunlight (the "photo" bit) and use it to make sugars (the "synthesis" part).

- The green pigment, **chlorophyll**, which absorbs sunlight, is located on the internal membranes of the **chloroplast**. These membranes are tightly folded so as to pack more light absorbing area into each chloroplast.

Chromosome Structure in Eukaryotes

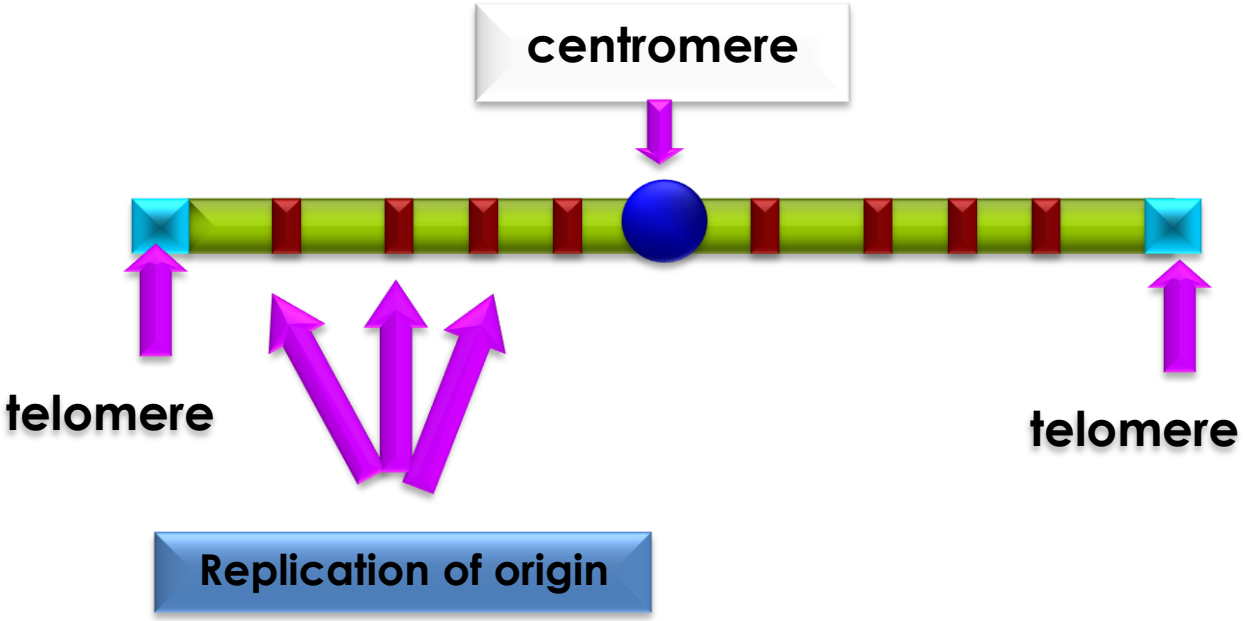
- Higher organisms have multiple chromosomes, whereas bacteria only have **one**. Moreover, unlike bacterial chromosomes which are circular, **eukaryotic chromosomes are linear**.

- This means they have ends and a middle. Both of these are special structures. The middle is called the **centromere** and although it is often more or less centrally located, it is sometimes closer to one of the ends.

- At cell division, when the chromosomes are replicated to give two copies, **microscopic fibers** (*microtubules*) are attached to the centromeres and drag the two sets of chromosomes apart.

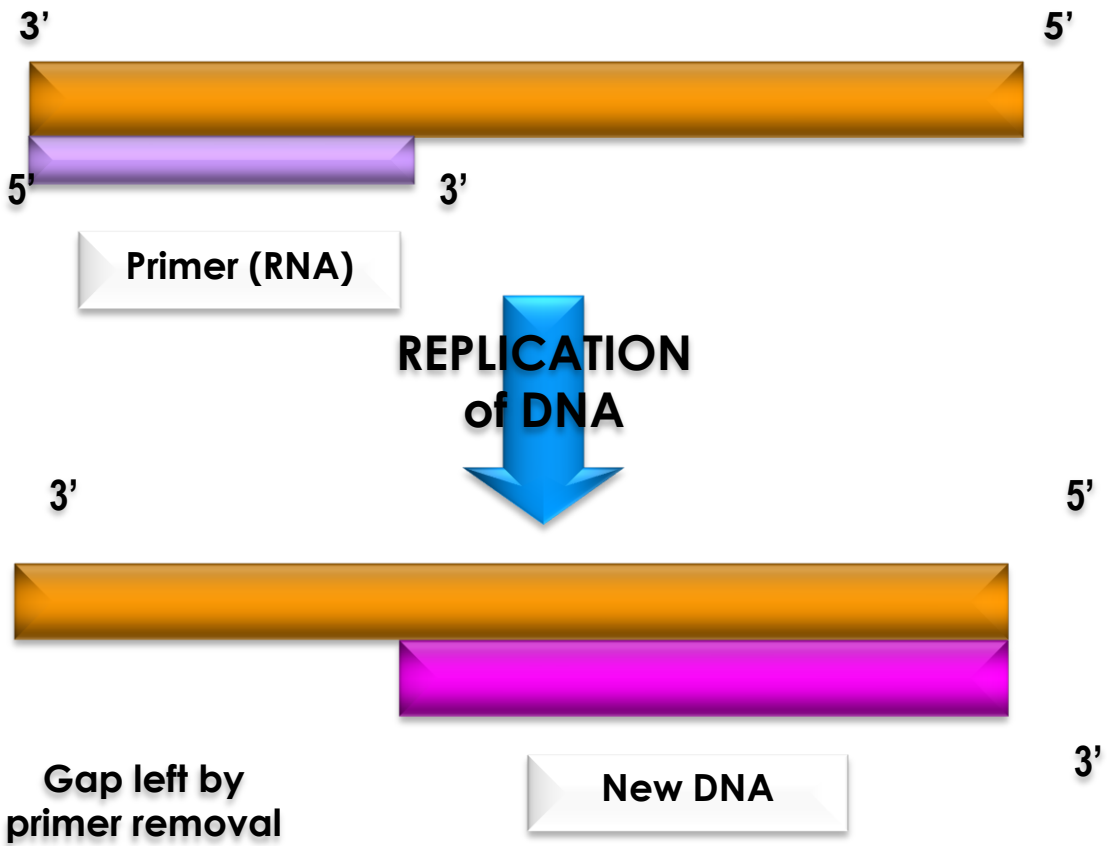
- The **end structures** of eukaryotic chromosomes are called **telomeres**.

General Eukaryotic Chromosome Structure

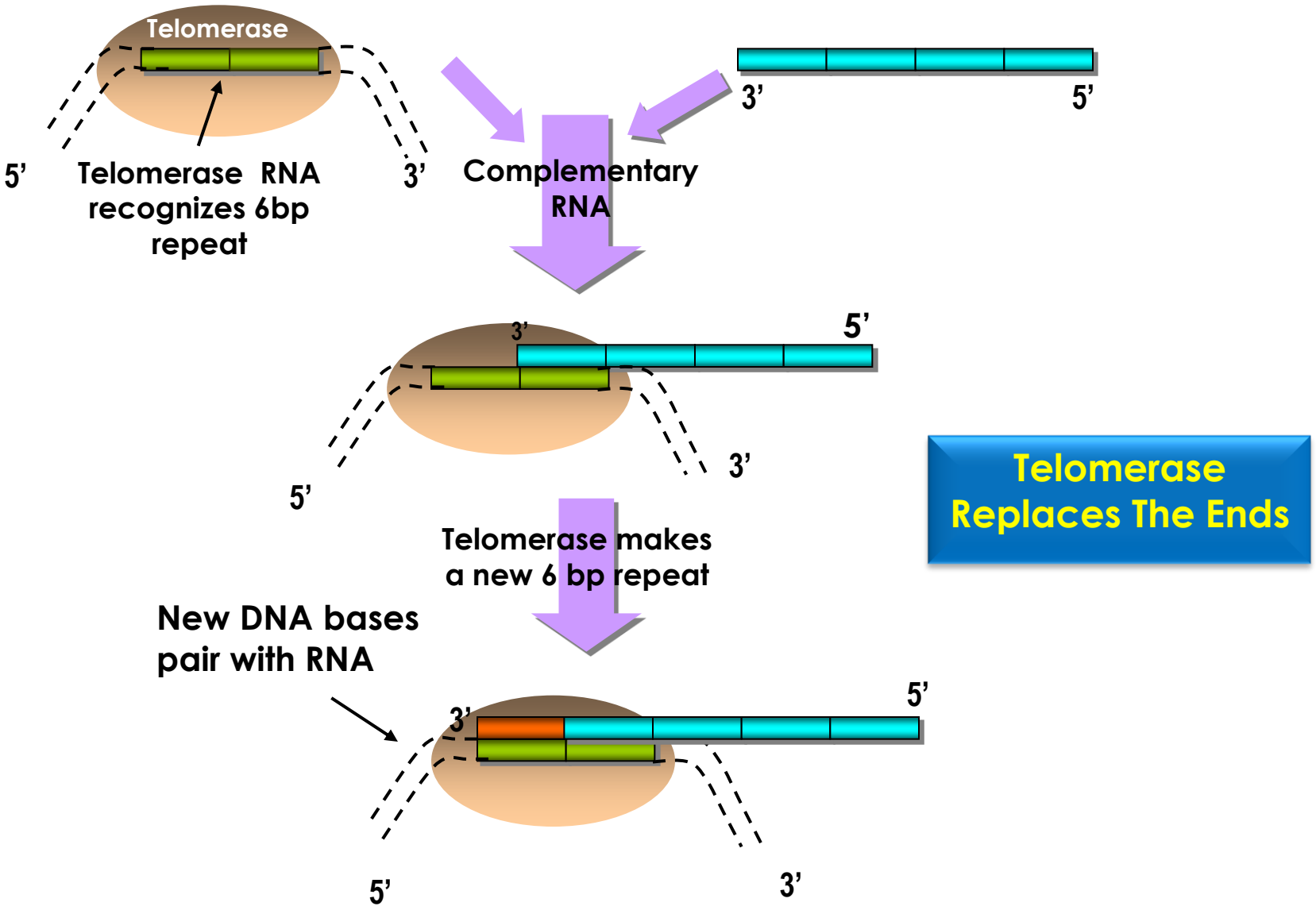


"The End is Nigh (near)" – Telomeres

- Whenever a DNA molecule is replicated, the process of making a new chain always starts with a primer made of RNA. This primer is later removed.
- If the DNA molecule is circular there is no problem, replication goes around the circle and fills from behind the gap where the primer used to be.
- If the DNA molecule is linear, there is a problem which must be overcome.
- A linear chromosome would get shorter, by one primer length, each time it is replicated. Eventually, if nothing was done, it would slowly disappear.


3'
5'
5'
3'
Primer (RNA)
REPLICATION
of DNA
3'
5'
3'
Gap left by
primer removal
New DNA

- The telomeres at the ends of eukaryotic chromosomes consist of a six base pair sequence repeated about 2,000 times.
- During each replication cycle the chromosomes are, in fact, shortened due to loss of the RNA primer.
- However, an enzyme known as **telomerase** cancels this loss out by adding a few of the six base pair chunks each time around.
- Presumably in honor of the lost RNA primer, telomerase carries around with it a small bit of RNA complementary to the six base pair telomere repeat. This allows it to recognize the telomeres, and reminds it what sequence to make.



Telomerase Replaces The Ends

Back up Your Files!!

- People often need to make a back-up copy of important documents especially if they have to submit the original! In the same way, most eukaryotic cells have a back-up copy of each gene.

- Since genes are carried on chromosomes, this means that they have pairs of duplicate chromosomes which are clearly seen when they line up with their partners at cell division. Human cells have **2 x 23 = 46 chromosomes**.

- A cell with only a single set of chromosomes and hence only a single copy of each of its genes, is known as **haploid**.

- Cells with duplicate copies of their genes are referred to as **diploid**. Bacteria have only a single copy of each of their genes. So if a vital gene is damaged, that whole cell containing the defect is doomed.

- On the other hand, **bacteria are small** and **divide rapidly** so they can afford to lose a few of their friends.

- **Eukaryotic cells** are much **larger** and **divide more slowly**. In other words, each cell represents a greater investment of time and resources, so keeping a back-up copy of each gene is a sensible policy.

How is DNA Packaged?

- Eukaryotic cells have vastly more DNA than lower organisms. Even though eukaryotic cells have 10 to 20 times as many genes as bacteria, most of their DNA does not even consist of genuine coding sequence.

- Some of this **extra DNA is found between genes**, whereas amazingly, other **non-coding DNA is actually inserted into the genes**.

- Since as much as **95 percent** of the DNA in a **eukaryotic cell may be non-coding**, it means that it may contain **500 times as much DNA as a bacterial cell**.

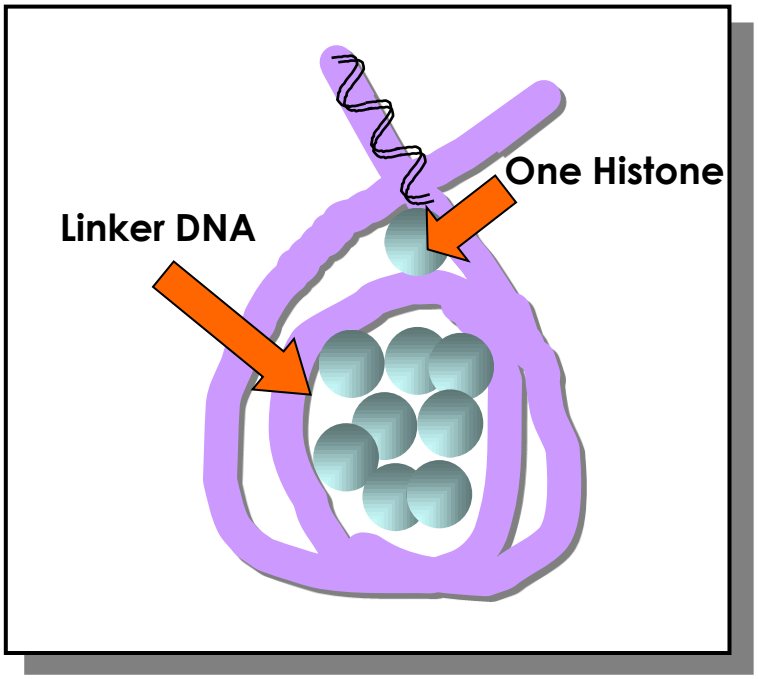
- This **eukaryotic DNA** is **stored on the chromosomes in the nucleus.** Each chromosome is a single molecule of DNA.

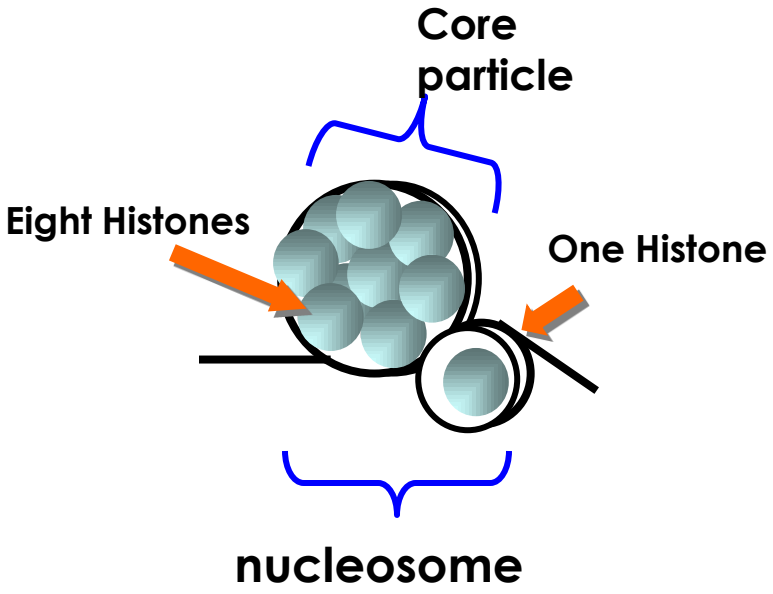
- **In bacteria**, there are approximately **3,000 genes on a single chromosome** which is about **1 millimeter long**.

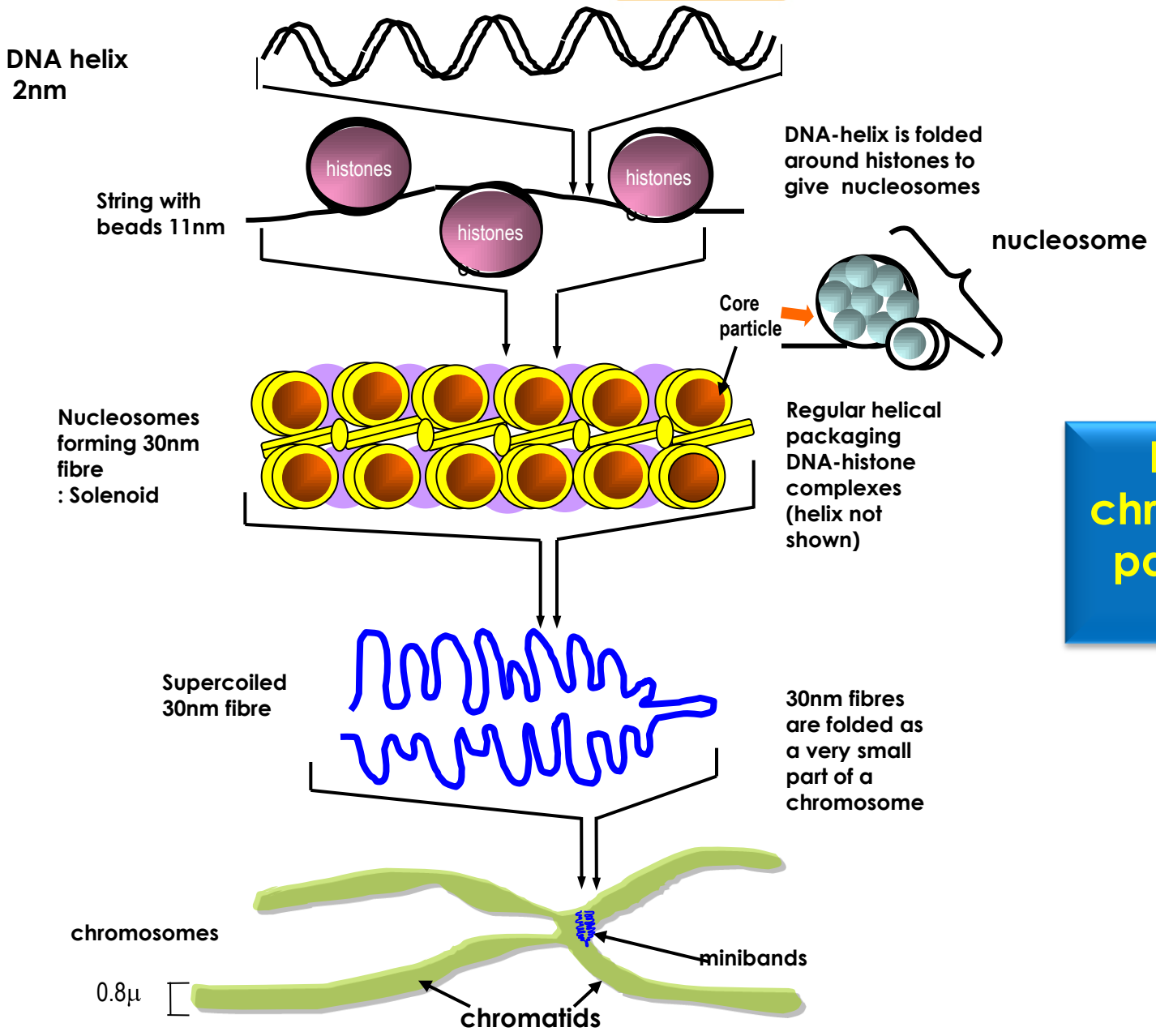
- Eukaryotic chromosomes are thus **1,000 times longer** than the bacterial cell in which they belong.

- Eukaryotic chromosomes may be as much **one centimeter long** and **must be folded up to fit into the cell nucleus** (which is **5 microns across**), a 2,000-fold shortening.

- The DNA starts folding by coiling around the **histones**, **positively charged proteins** that neutralize the negative charge of the DNA itself.
- DNA with histones bound to it was named **chromatin** when it was first discovered in chromosomes.
- Each 200 base pairs of DNA is wrapped around nine histone proteins forming a **nucleosome**.
- Eight of histones cluster together and 140 base pairs of DNA are coiled around them, so forming a **core particle.**


Linker DNA
One Histone


Core particle
Eight Histones
One Histone
nucleosome



Level of chromosome packaging

- A **linker region** consisting of the remaining **60 bp of DNA** and the ninth histone molecule join each core particle to the next.

- Overall, a chromosome with its DNA twisted into a series of nucleosomes would resemble a **string of beads**; accept that the folding process continues.

- The **chain of nucleosomes** is **wound helically into a giant solenoid structure** with **six nucleosomes per turn**, the 30 nanometer fiber.

- In turn these fibers are looped back and forth. There are about **50 of the solenoidal turns per loop** and the ends of the loops are attached to a protein scaffold.

- **Eighteen of these loops** are then wound **radially around the chromosome** axis to give a **miniband**.

- Roughly **a million such minibands** form a complete chromosome.

SUMMARY OF CHROMOSOME FOLDING

Level of folding	Consists of	Basepairs per turn
DNA double helix	nucleotides	10
Nucleosomes	200 base pairs each	100
30nm fibre	6 nucleosomes per turn	1 200
Loops	50 solenoid turn per loop	60 000
Chromatid	1million minibands	1 080 000

- When chromosomes are visible under the microscope it is because they have been caught in the act of dividing.

- Before cell division and during normal cell operations, the DNA is spread out for use in transcribing genes.

- At this point the chromosome consists of a single molecule of double helical DNA. In this state, it does not look at all like typical chromosome pictures.

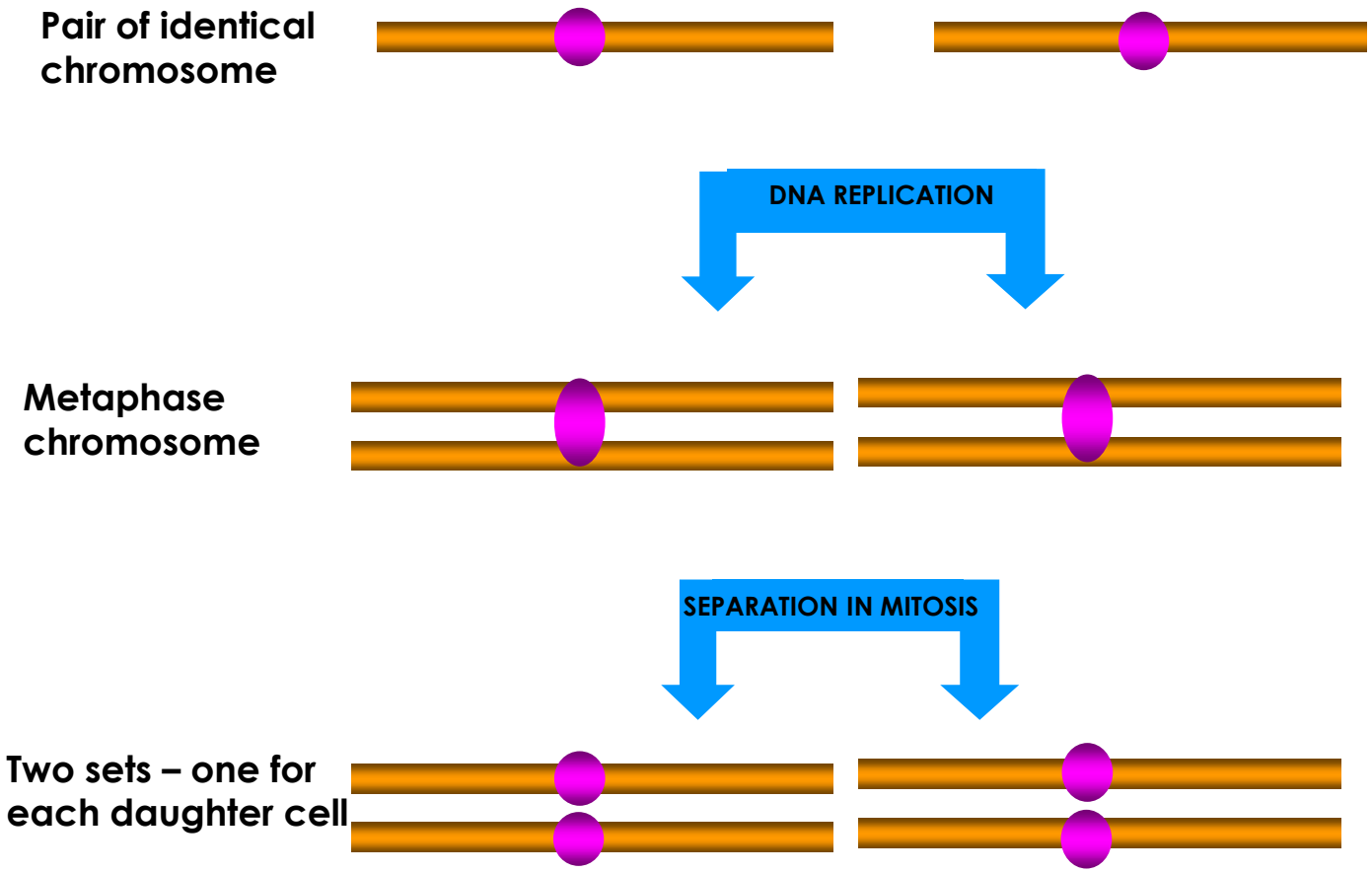
- Just before cell division, the **DNA condenses** and is folded up tightly enough so it becomes much easier to see.

- The typical metaphase chromosome, seen in most pictures, has just duplicated its DNA and therefore consists of two identical DNA molecules still held together by the centromere.

- These duplicate strands of DNA are known as **chromatids**. This metaphase chromosome is just about to divide into two daughter chromosomes.

- But remember that in a **non-dividing cell**, the chromosomes only **have a single chromatid**.

Interphase And Metaphase Chromosomes



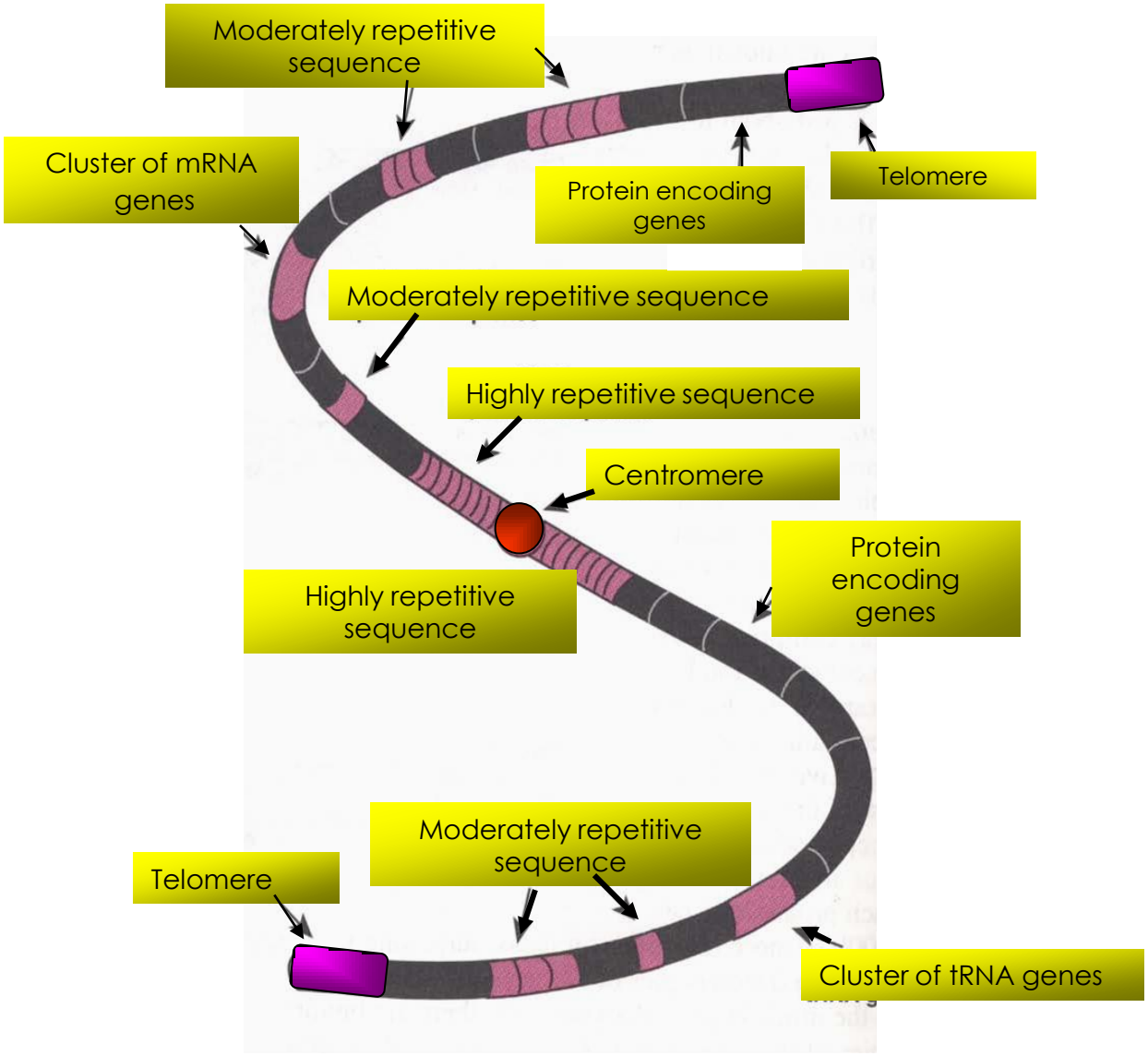
Repeated Sequences

- Most genes are present only in single copies. Such unique sequences account for almost all bacterial DNA. However, in higher organisms, **unique sequences** may comprise as little as 20 percent of the total DNA.

- For example, we **humans have 65 percent unique DNA**, whereas **frogs have only 22 percent**. The rest of the DNA is made up of repeated sequences of one kind or another (See **Stlylised Eukaryotic Chromosome**).

- Since **each prokaryotic cell contains 10,000 or more ribosomes**, it is not surprising that **their DNA usually contains half a dozen copies of the genes** for ribosomal RNA and transfer RNA.

Stylised Eukaryotic Chromosome



Gene Structure in Eukaryotes

- Not only is useless DNA scattered throughout the eukaryotic chromosomes between the genes, but the actual genes themselves are often interrupted with **non-coding DNA**.

- These intervening sequences are known as **introns** and the regions of the DNA which contains coding information are known as **exon**.

- Most eukaryotic genes consist of exons alternating with introns. In lower, single-celled eukaryotes, the introns present are relatively rare and often quite short.

EXONS AND INTRONS

- In contrast, in higher eukaryotes, most genes have introns and they are often longer than the exons.
- In some genes, introns may occupy 90 percent or more of the DNA.

Transcription in Eukaryotes

- Since eukaryotic cells have l0 times as many genes as bacterial cells, deciding which to turn on, and when, is much more complicated.
- Frequently the whole business of transcription is more complex.
- For a start, eukaryotes have three **different RNA polymerases**, unlike bacteria which have just one. The "union" rules for RNA polymerase are as follows.

RNA Polymerase No:	Genes Transcribed:
I	Genes for large ribosomal RNAs
II	Genes which code for proteins
III	Genes for tRNA, 5S and a few tiny RNAs

- Since **ribosomal RNA and transfer RNA** are **needed all the time** and in types of cells, the **genes** encoding them are **regarded housekeeping genes** and **RNA polymerases I and III** go about their business pretty much automatically.

- In contrast, **RNA polymerase II** actually has to think about what it is doing.

- In a multicellular organism, different cell types produce different types of proteins.

Different Cell Types of Multicellular Organism

- For example, red blood cells produce hemoglobin, whereas white blood cells make antibodies. Worse still, protein production often varies during development. Fetal hemoglobin is different from the adult version.

- The activity of RNA polymerase II is **regulated** by a large number of *accessory proteins,* called **transcription factors** that bind to and **recognize specific sequences** on the DNA. These DNA sequences are of two major classes, the **promoter and enhancers**.

Promoters of Eukaryotes

- **Promoters** are found in front of all genes, both prokaryotic and eukaryotic. A eukaryotic promoter consists of three regions:

A. The **initiator box** is a sequence found at the site where **transcription starts.**

B. About 25 base pairs upstream from this is the **TATA box**, an AT-rich sequence which is recognized by a protein, imaginatively called the **TATA box factor**.

RNA polymerase II, the **TATA box factor** and **some other proteins** stay together as a bulky complex sometimes known as the **"transcription apparatus".** See figure "**PROMOTER LAY-OUT IN EUKARYOTES**"

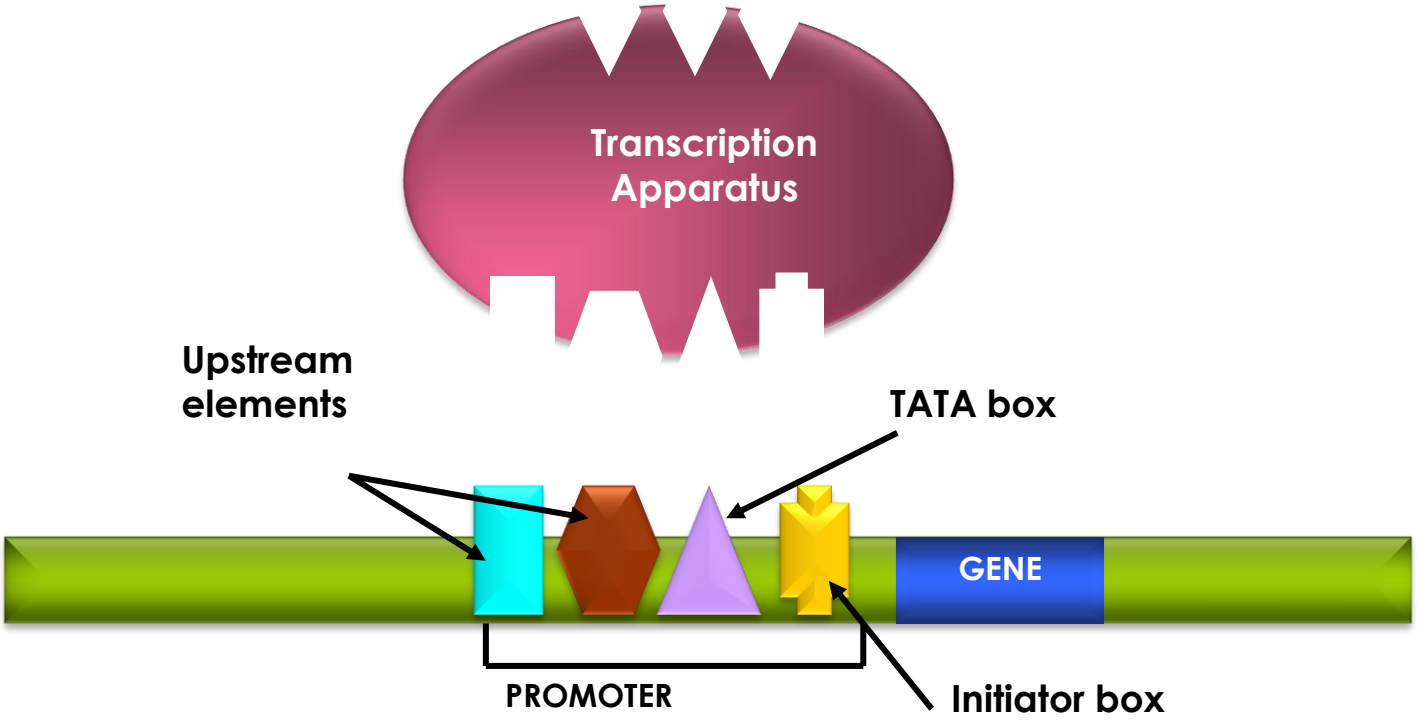
Third Component of the Eukaryotic Promoter

C. The third component of the promoter is the **upstream element**. There are many different upstream elements.

They are about **10 base pair long** and are recognized by specific proteins. In fact, depending on the gene, there may be more than one upstream element in a given promoter.

The more upstream elements present, the more complex the control of transcription.

Promoter Layout In Eukaryotes



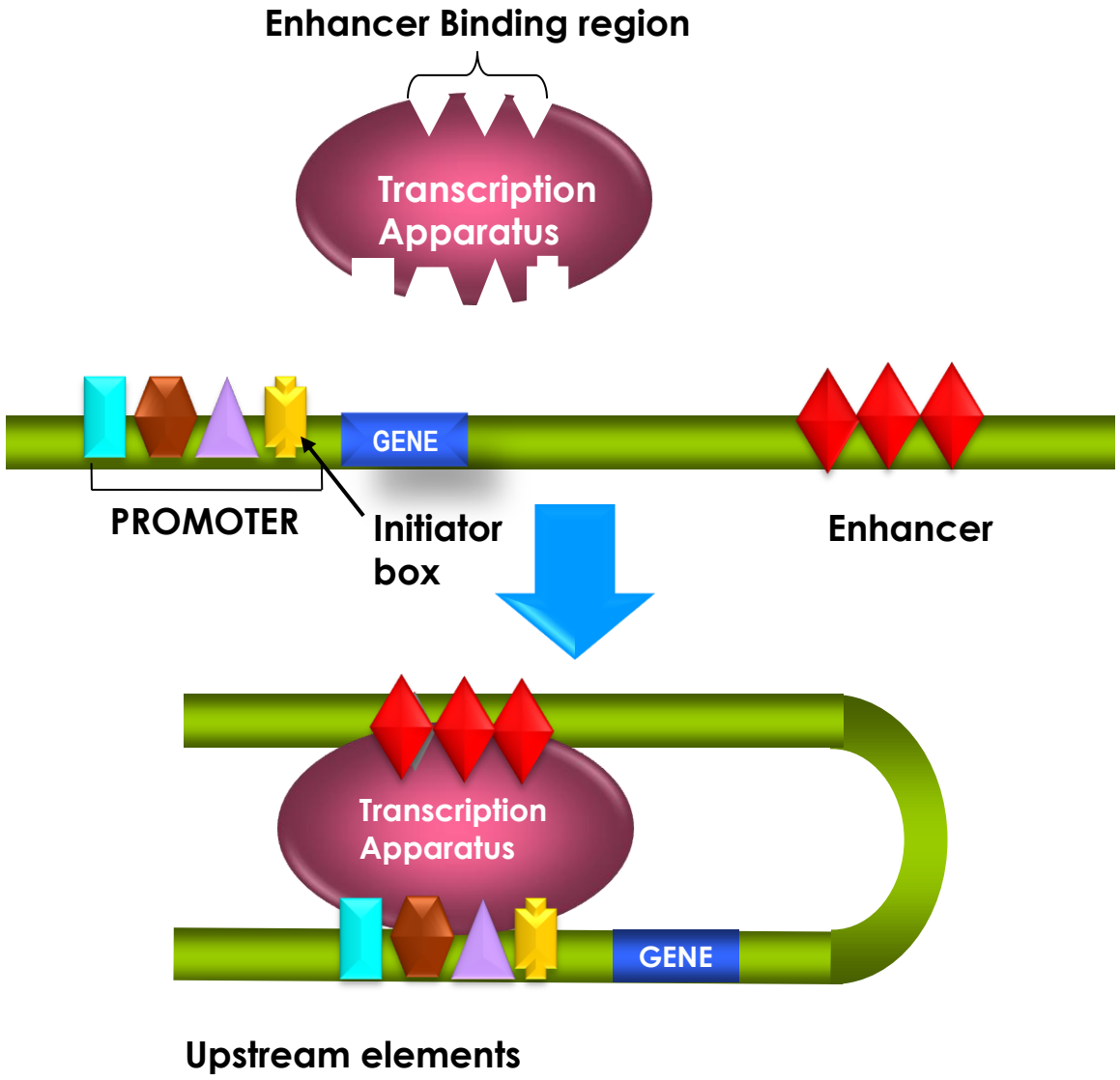
Enhancers

- **Enhancers** are sequences which are involved in gene regulation, especially during development or in different cell types.

- Enhancers do exactly what their name indicates, they **enhance the rate of transcription** as a result of binding certain specific transcription factors.

- Although enhancers are sometimes found close to the genes they control, more often they lie at some considerable distance perhaps thousands of base pairs away. Even odder is that they may be located either **upstream or downstream from the promoter**.

- When an enhancer switches a gene on, the DNA between it and the promoter loops out as shown in the next figure (**ENHANCER ACTION INVOLVES LOOPING OF DNA**).



Enhancer action involves looping of DNA

Transcription Factors

- These are **specialized proteins** that **regulate gene expression** by **controlling transcription**. They have **four domains** needed for the following functions:

  1) **binding to a specific sequence** on the DNA

  2) **binding to the RNA polymerase II** complex

  3) **getting into the nucleus** where the genes are kept,

  4) **responding to a stimulus** of some sort which should be turned on.

- An example of a transcription factor is **MyoD** which only appears in those cells destined to become muscle cells.

- The MyoD factor switches on a wide selection of genes needed in muscle cells, but is not required in other cell types.

- A special class of transcription factors is needed for the development of embryos into adults in higher organisms.

RNA Processing

- You might think now that RNA polymerase has done its work of transcribing the gene, we have our messenger RNA and we can zoom off to the ribosome and get translated into protein.

- Not so fast. For one thing, transcription has just taken place using the DNA which is **inside the nucleus**, whereas the **ribosomes are outside**!

- Far, far worse, however, is that most eukaryotic genes have those useless intervening sequences, the **introns**.

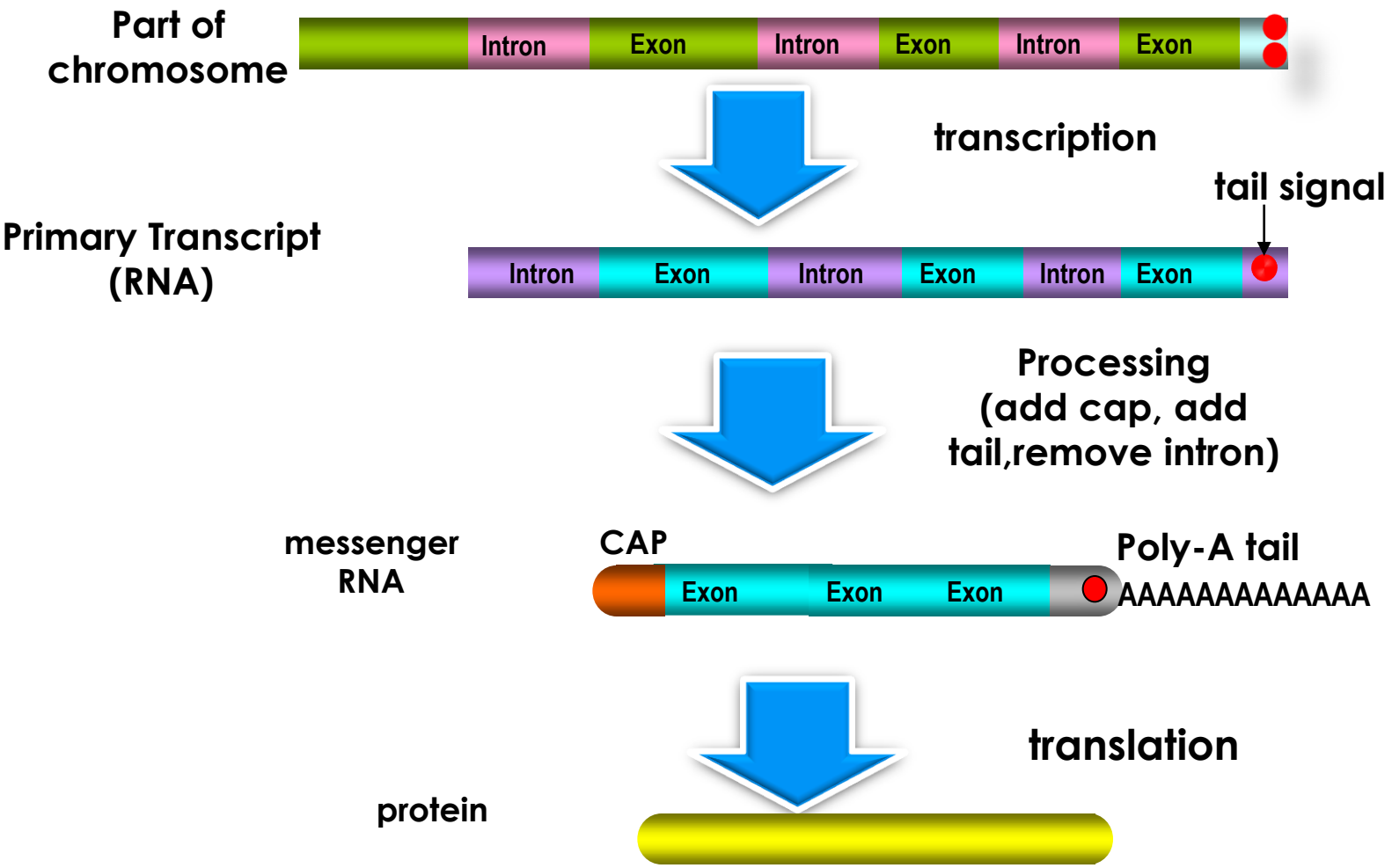
- Thus the **DNA sequence of eukaryotic gene** consists of regions which code for part of the final protein, the **exons**, alternating with the regions of non-coding DNA, the **introns**.

- The RNA molecule resulting from transcription is known as the **primary transcript**. It is not actually genuine certified messenger RNA because the **primary transcript**, too, **has exons alternating with introns**.

- If we translate the primary transcript we would get a huge dysfunctional protein with lots of extra stretches of nonsense (useless protein).

- In fact the primary transcript is trapped inside the nucleus until the introns are removed.
- This is known as **splicing** and involves **cutting out the introns** and **joining the ends** to generate an RNA molecule which has only the exons.
- In fact, as shown it contains an uninterrupted coding sequence. In order to be recognized as a true messenger RNA molecule, **two other modifications** must be made.
- These are the addition of a cap structure to the front and a tail to the rear of the RNA molecule. In fact, as shown in below, these are added before splicing out the introns.

Expressing A Eukaryotic Gene



Capping and Tailing

- Just as a college graduate receives a cap and gown before leaving the university to earn money in the real world, so too, messenger RNA must **be capped and tailed** before being allowed to leave the nucleus.

- RNA molecules destined to become messenger RNA, have a cap added to their 5' ends and a tail added to their 3' ends. This occurs **inside the nucleus** and **before splicing**.

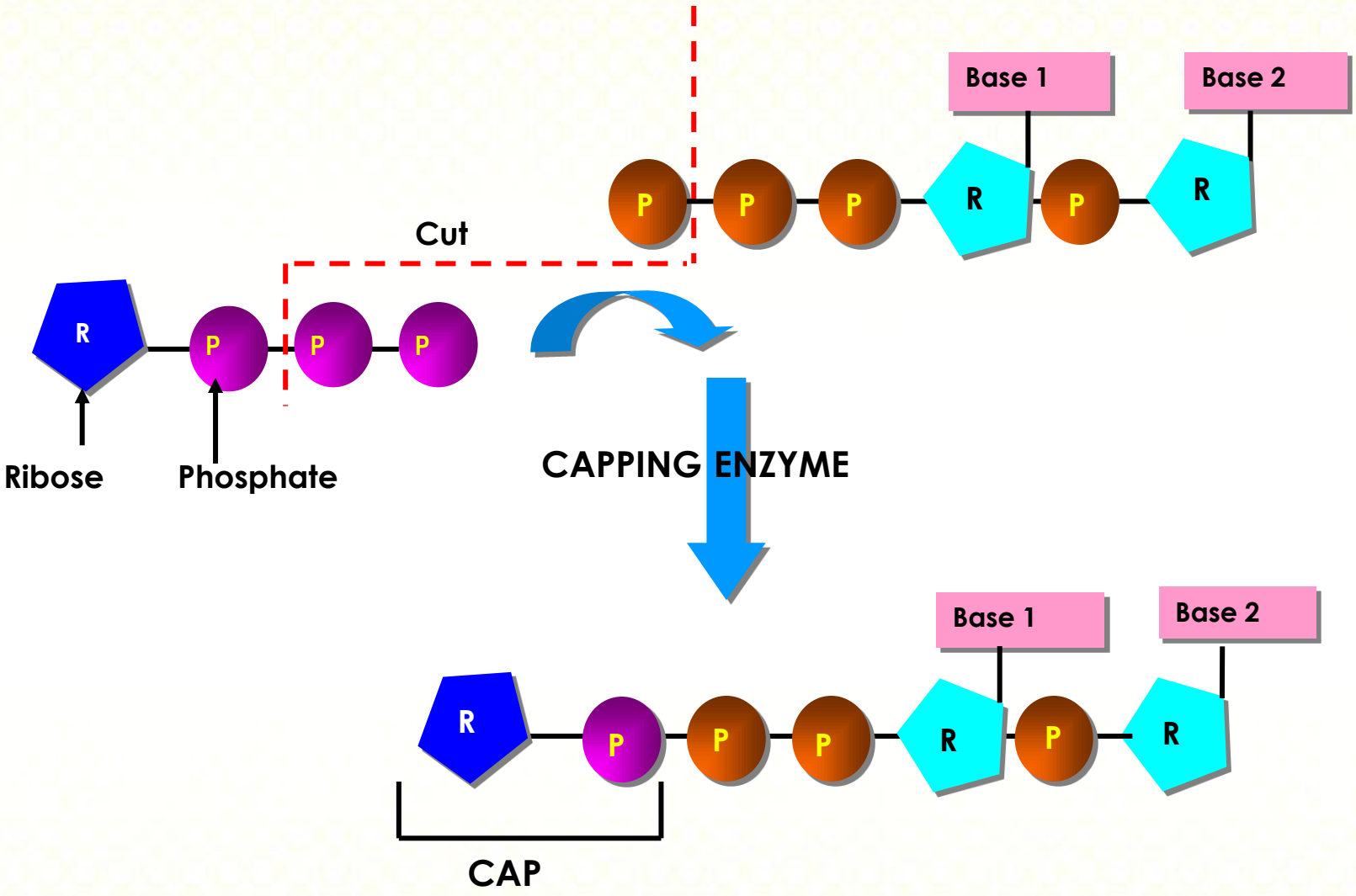
- Shortly after transcription starts, the 5' end of the growing RNA molecule is capped by the addition of a **guanosine monophosphate** (GMP) (**see diagram ADDITION OF CAP EUKARYOTIC RNA**).

- This is added in a **backwards orientation (3'-5')** relative to the rest of the bases in the RNA.

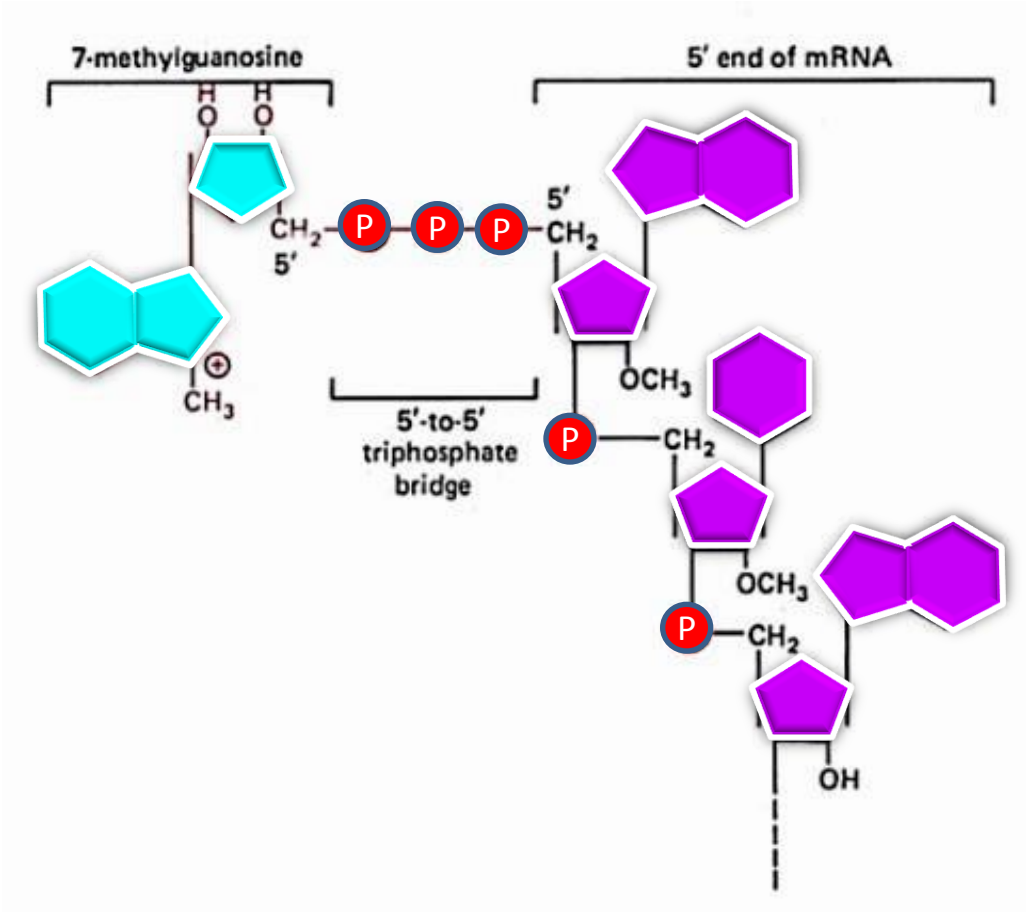
- After the addition of the GMP, the guanine base has a **methyl group** attached.

- **Extra methyl groups** may be added to the ribose sugars of the first or two nucleosides of the RNA in some higher organisms.

Addition Of Cap Eukaryotic RNA



Composition of the eukaryotic 5' "CAP"



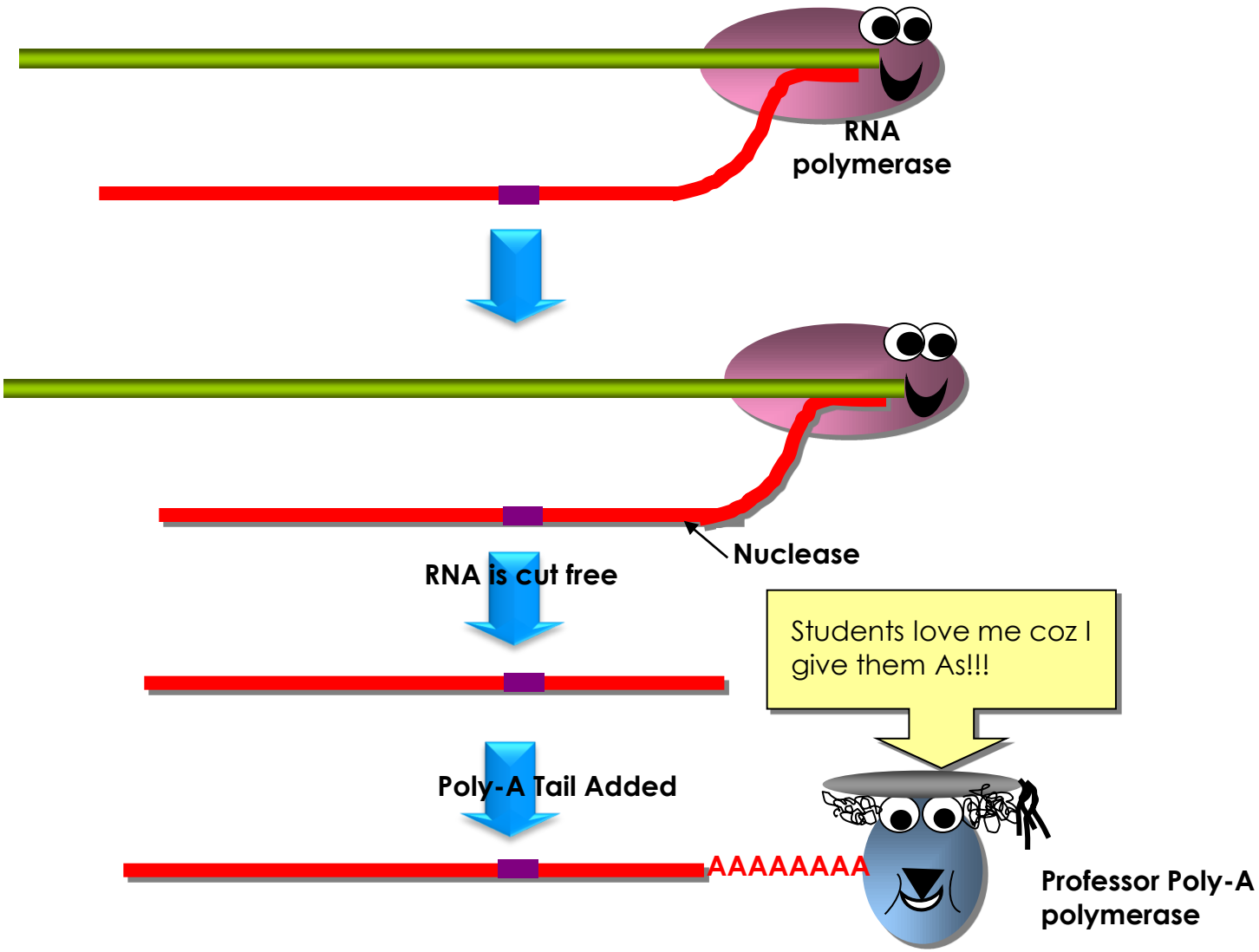
In eukaryotes the 5' end of the mRNA is coupled via a triphosphate bridge to methyl guanine residue. The terminal nucleotides are also often methylated at the 2' hydroxyl position on the ribose moiety. The net result is to render the 5' end of the mRNA highly positively charged.

- After being capped, the growing RNA is tailed (see next diagram - **Addition Of POLY – A Tail**). There is a recognition sequence - **AAUAAA** - at the 3' end.

- The RNA polymerase which is making the RNA molecule cruises on past this point (**AAUAAA**). However, **a specific endonuclease** recognizes this sequence and cuts the growing RNA molecule 10 to 30 bases downstream.

- The enzyme **polymerase** now comes along and adds a run of **100 to 200 adenine residues** to form the tail.

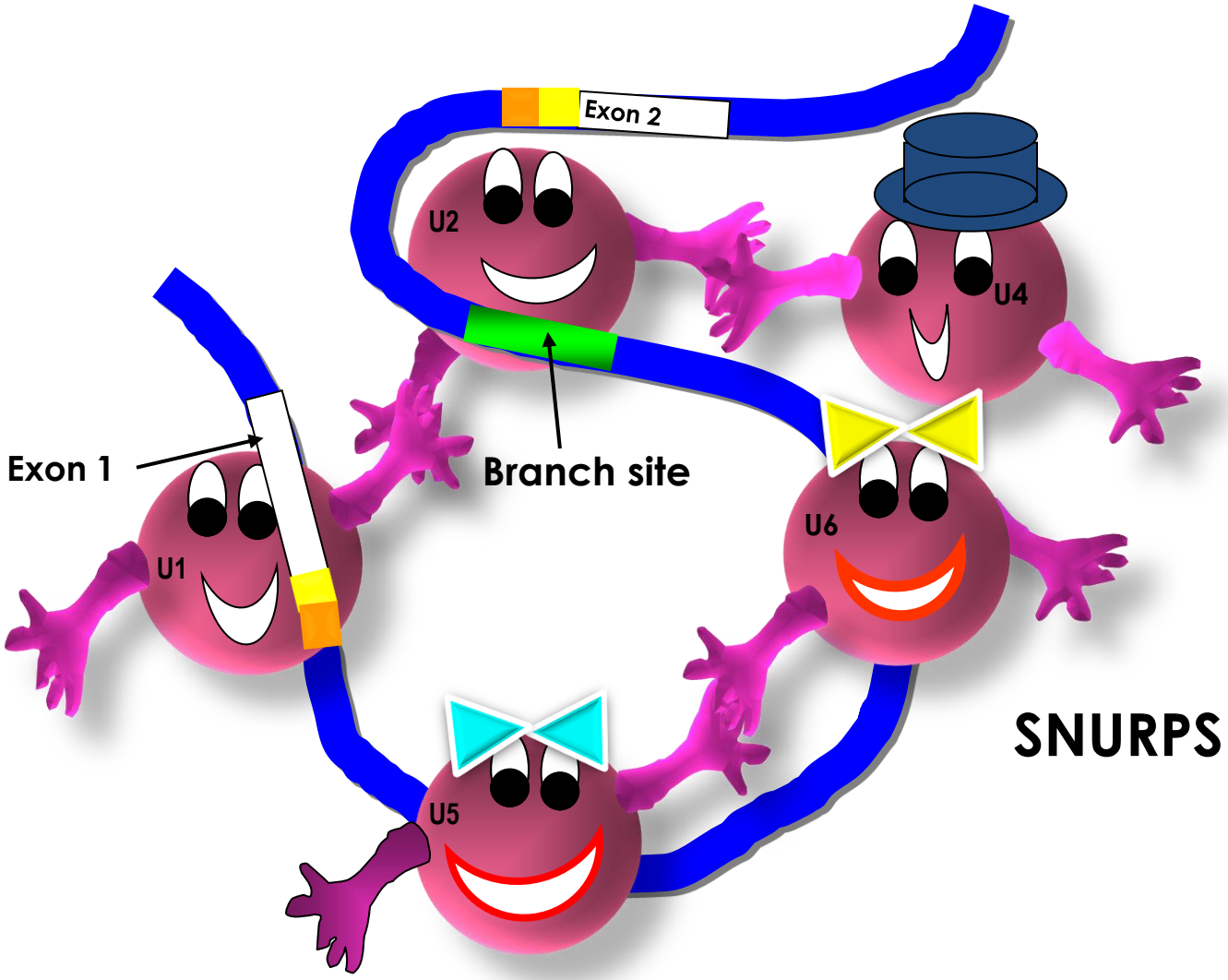
Addition of Poly – A Tail



RNA Splicing

- The next step in the processing of the RNA is the **splicing out of the introns**.
- The splicing machinery is known as the **spliceosome** and consist of **several proteins** and some specialized, **small RNA molecule** found only in the nucleus.
- Each "SnRNA" plus its protein partners forms a **small nuclear ribonucleoprotein (SnRNP).**
- There are five SNURP's - numbered from **UI to U6** (with U3 missing). You can picture them as little elves, sorry, I mean snurps, working together to splice RNA.

RNA Splicing – The Spliceosome

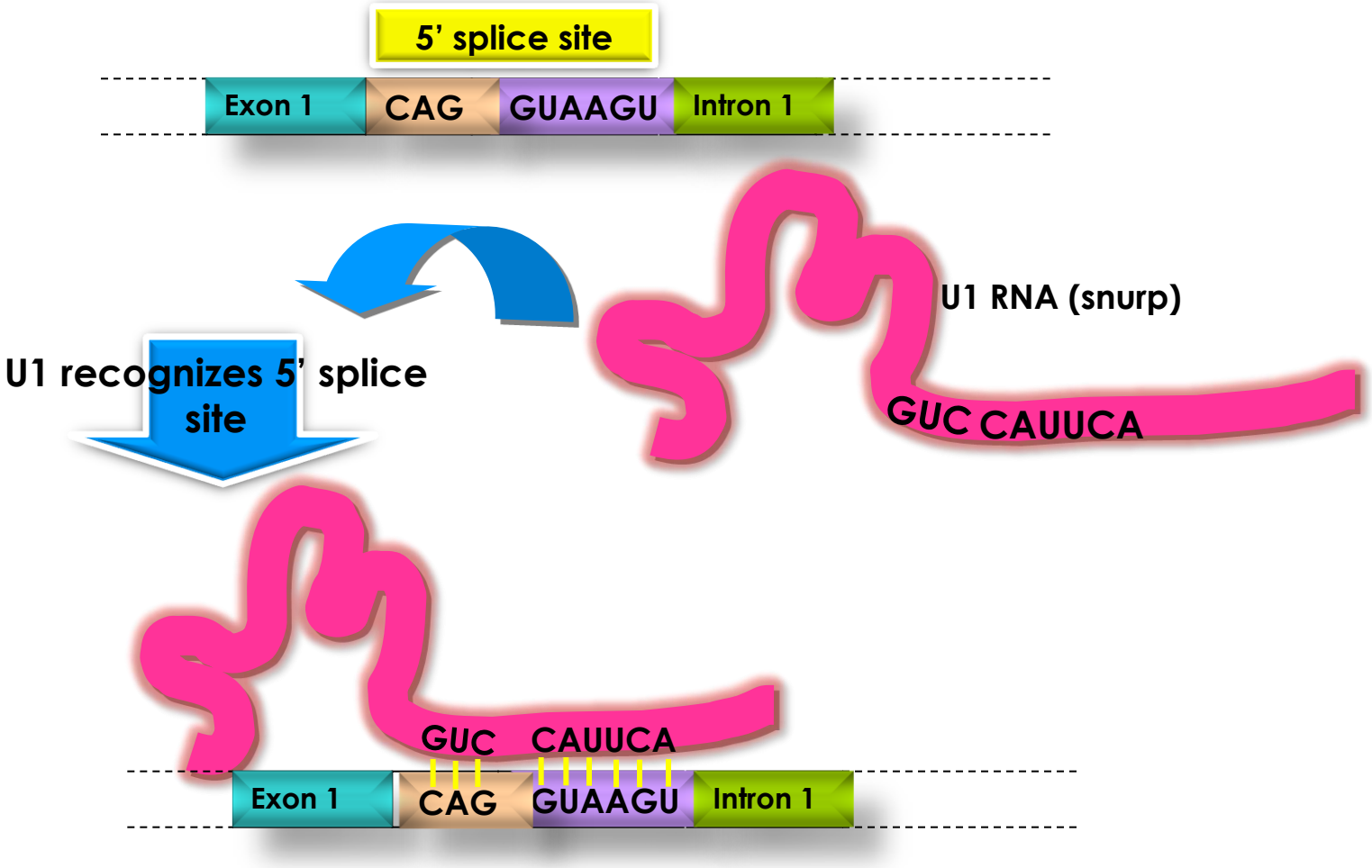


- During the first stage of splicing, the spliceosome recognizes both ends of the intron and binds to them. This makes the **intron RNA loop out** as shown in the previous page.

- So why do the snurps **need both RNA and protein**? They use their **small RNA molecules** for the task of **recognizing the splice and branch sites** on the larger RNA molecule they are processing.

- As you would imagine, this is done **by base pairing**. The **protein part** of the snurp then does the manual labor of **cutting and sticking** as would an enzyme. This is illustrated below showing the **U I snurp** which **recognizes the 5' splice site**.

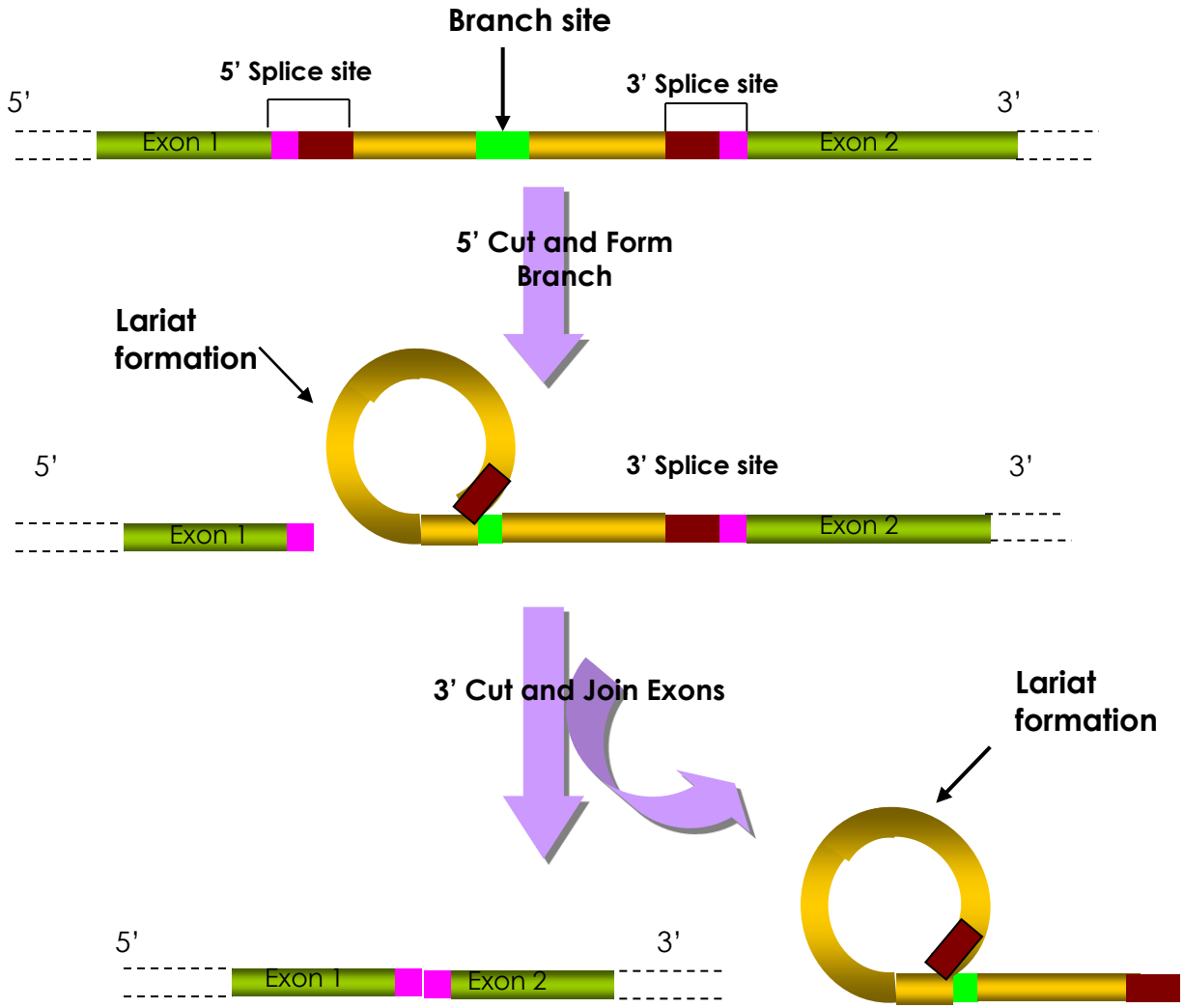
Snurp U1 Recognizes Splice Site



- **Splicing must be accurate** to within a single base since a mistake would throw the whole coding sequence out of frame and totally scramble the protein eventually, resulting from translation of the mRNA.

- The overall result of all this cutting and pasting is shown in **SPLICING – THE OVERALL SCHEME.**

Splicing – The Overall Scheme



Alternative Splicing

- Although any particular junction splice must be made with **total precision**, eukaryotic cells can sometimes choose to use different splice sites within the same gene.

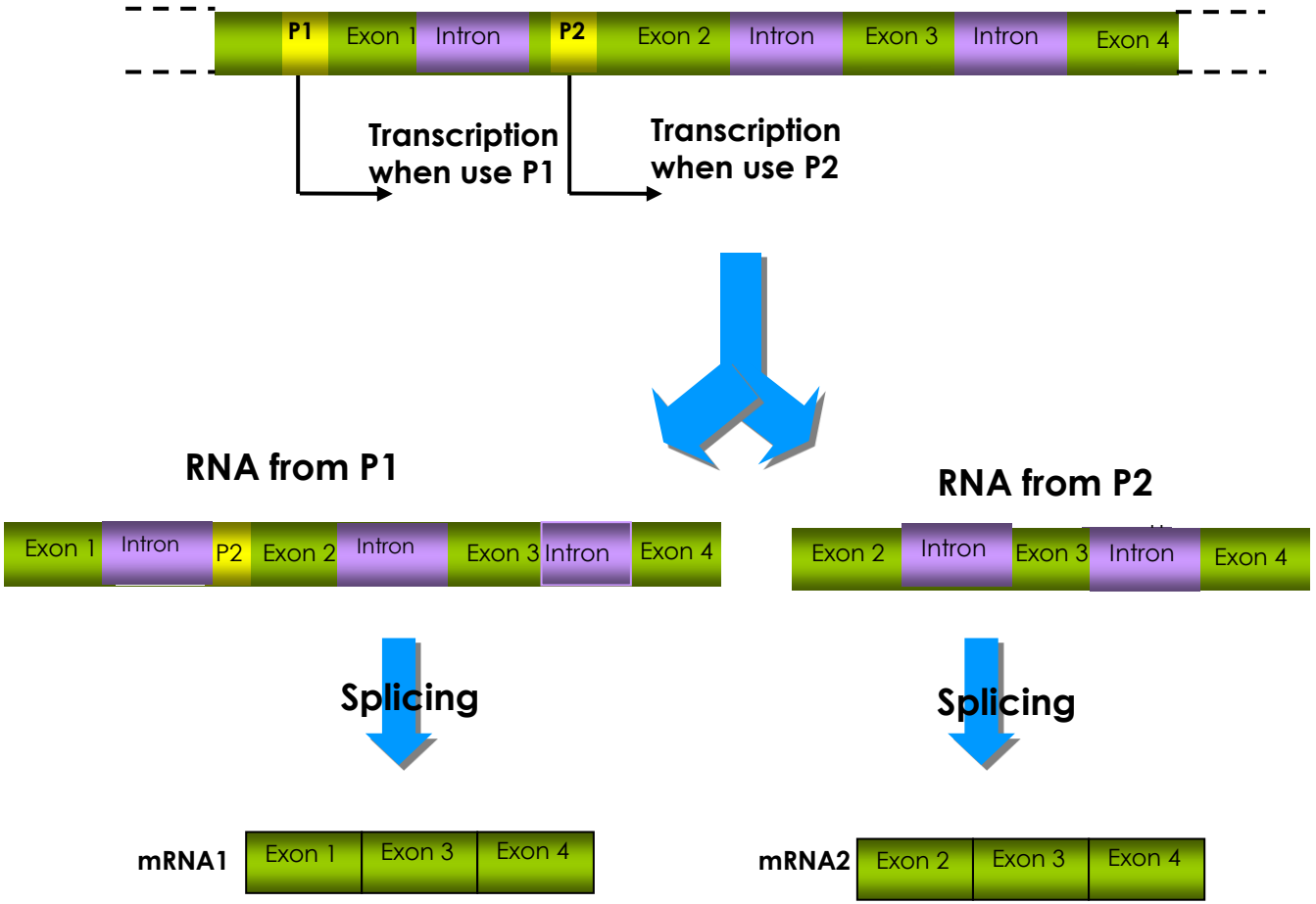
- Generally, splicing is used by different types within the same animal. This allows a **single original DNA sequence** to be used **to make several different proteins** which have distinct overlapping functions.

- There are **FOUR main types** of alternative splicing:

FIRST: Alternative Promoter Selection

- In this case, two alternative promoters are possible. The choice between them depends on **cell-type specific transcription factors**.
- Note that in this case there are **two alternative primary transcripts**.
- If **promoter PI** is used, then the sequence containing **P2** and **exon-2** is **spliced out**.
- If **promoter P2** is used, then **exon No. I** is **not even part of the primary transcript** and is therefore **not in the mRNA**.

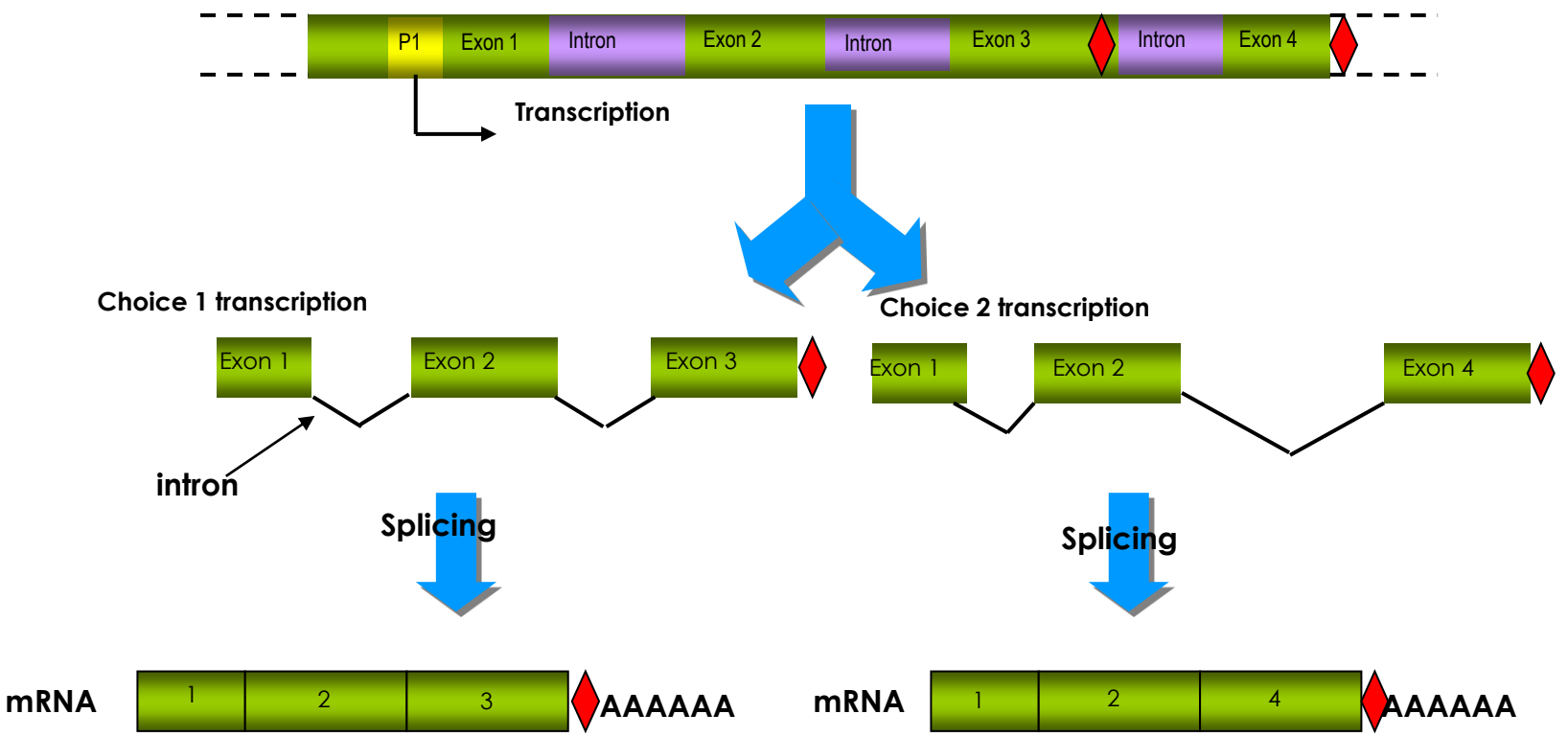
Alternative Promoter Selection



SECOND: Alternative Tail Site Selection

- **Two alternative sites** for adding the poly A tail may be possible. The choice between them again depends on cell type.
- In this case, **cleavage at the earlier poly A site** results in **loss of the distal exon**.
- If the **later poly A site** is chosen, then the **earlier poly A site** and the **exon just in front of it** are **spliced out**.
- This mechanism is used to produce antibodies which recognize the same invading, foreign molecule but which have **different back ends**. One type of antibody is secreted into blood, whereas the other type remains attached to the cell surface.

Alternative Tail Site Selection



THIRD: Alternative Splicing by Exon Cassette Selection

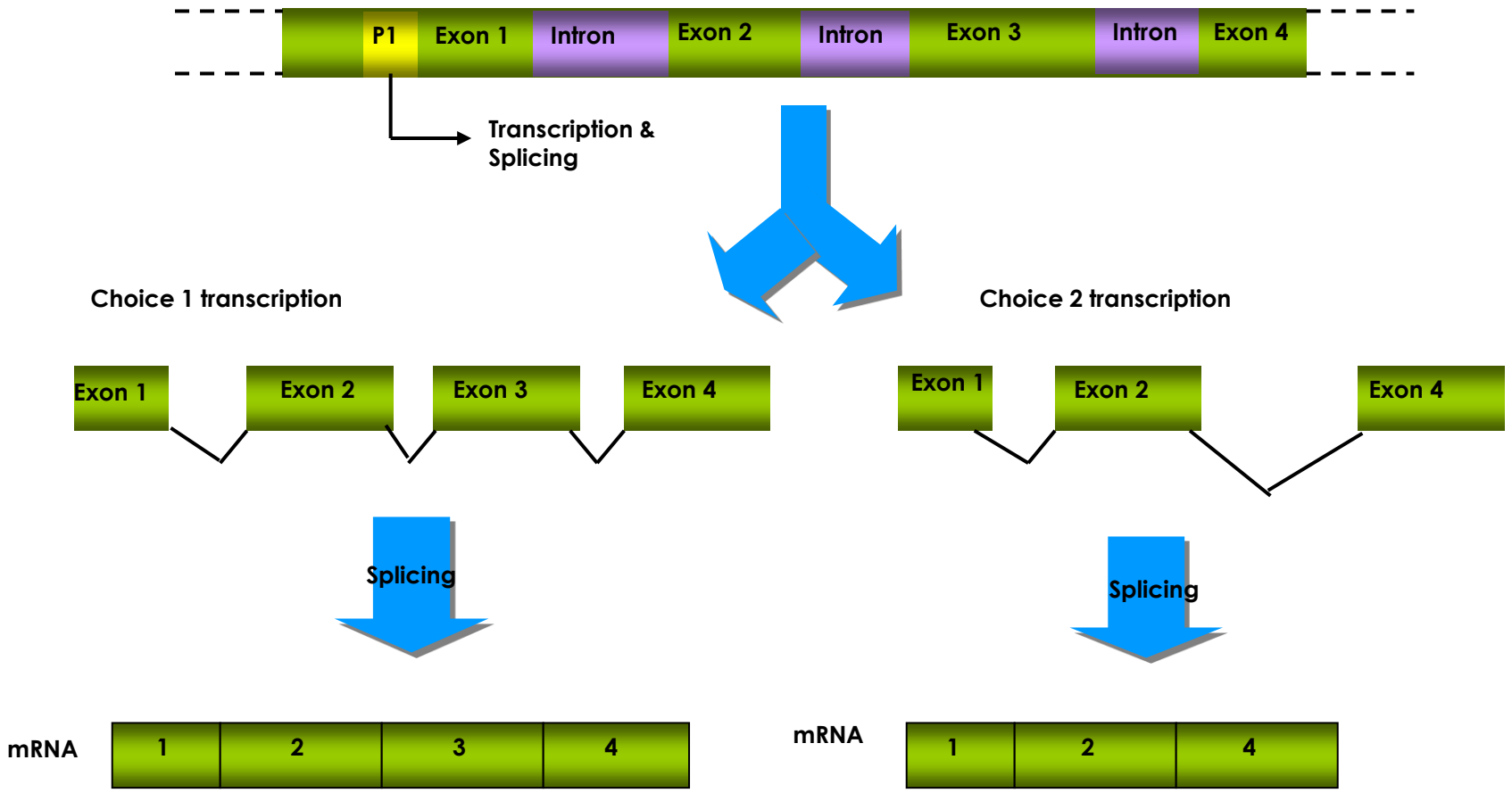
- Here we have a genuine choice of the actual splicing site. Depending on the choice made, **a particular exon may or may not appear** in the final product as shown in the next diagram.

- Here the **primary transcripts** are actually the **same**. They are drawn differently to illustrate the splicing plans.

- Some cell specific factor which recognizes the different possible splice sites must come into play here, but the details are still not known.

- Exon cassette selection occurs in the gene for the skeletal muscle protein, troponin T. In the rat this gene has l8 exons.

Principle of Exon Cassette Selection



- Of these, 11 are always used. Five (exons No. 4 through No. 8) may be used in any combination (including "none of the above") and the final two exons No. 16 and No. 17 are mutually exclusive, and one or the other must be chosen.

- This gives a mind-boggling **64 possible final messenger RNAs**. The result is that muscle tissue contains multiple forms of this, and other structural proteins.

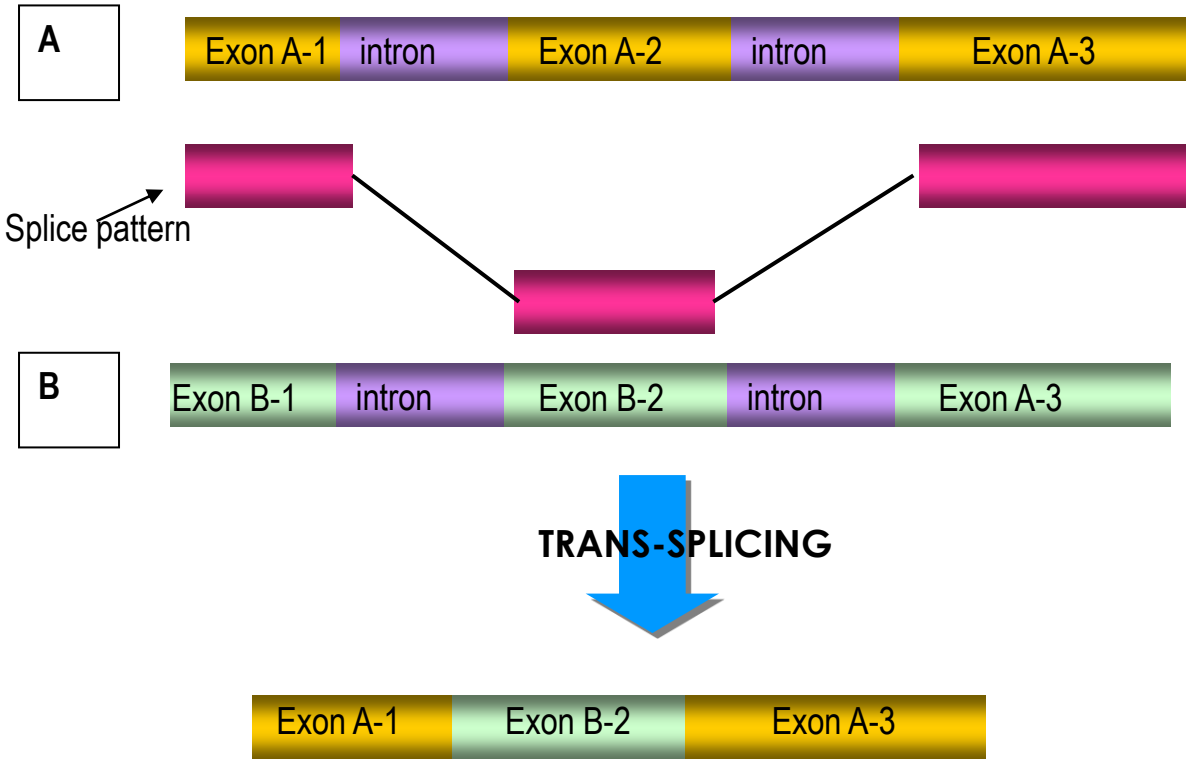
FOURTH: Trans-Splicing

- Until now we have spliced together segments of the same gene. Just as well, you probably think.

- Alternative splice sites **within a single gene** are quite confusing enough. Sewing random bits of one gene into the sequence for another would surely cause total confusion.

- Trypanosomes are parasitic single-celled eukaryotes that cause sleeping sickness and other awful diseases.

Trans-splicing



How do trypanosome escape detection?

- They **are not detected by the immune system** by constantly **changing the proteins on their cell surfaces** the dirty **genetic trick of shuffling gene**. In addition, they specialize in trans-splicing of many genes.

- On the other hand, trypanosomes do not appear to have introns and so do not have normal splicing although it has not (yet!) been found in higher animals, trans-splicing of segments from one RNA molecule into another also occurs in certain primitive worms, the nematodes, and in the chloroplasts of plant cells.

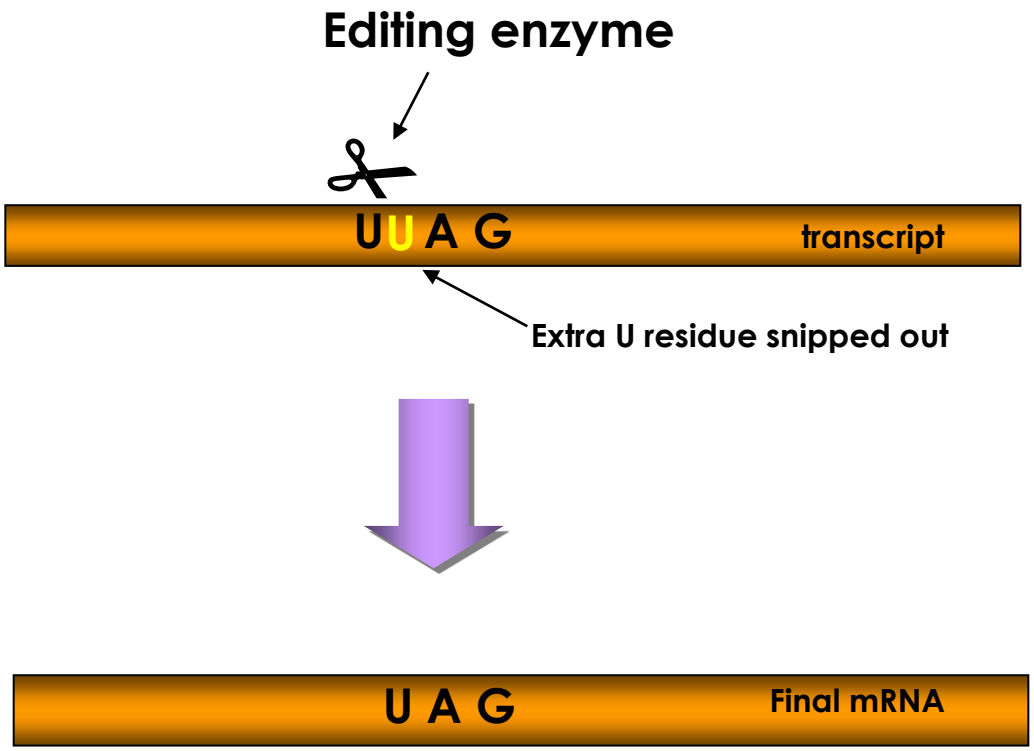
RNA Editing (*Penyuntingan RNA*)

- You thought the weird stuff was over. But no, it gets kinkier. Even more bizarre is **RNA editing**.

- Those sneaky enemies, the trypanosomes, are into this too. Some of the primary transcripts of trypanosomes are altered by **insertion or removal of uridine nucleotides**, one at a time before the final messenger RNA is generated.

- If the trypanosome did not edit its RNA, the result would be a **defective**, **frameshifted protein** made from an out of phase mRNA.

RNA Editing in Trypansomes

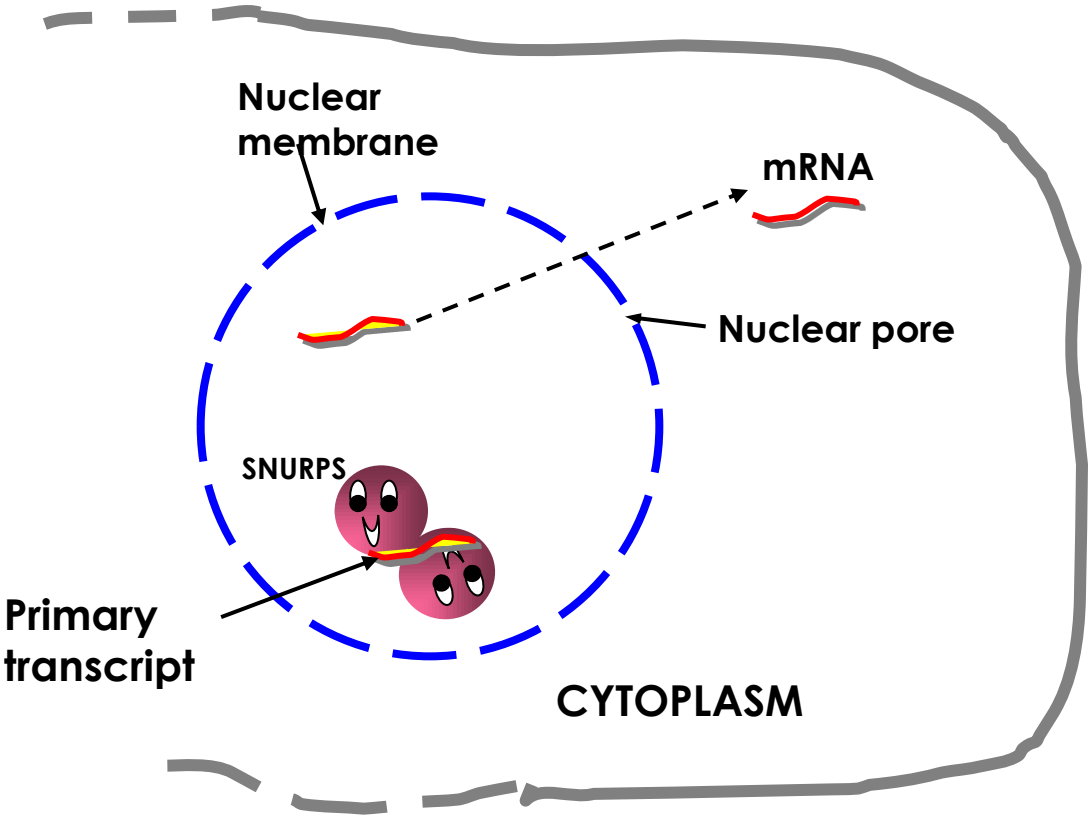


Transport Out of the Nucleus Membrane

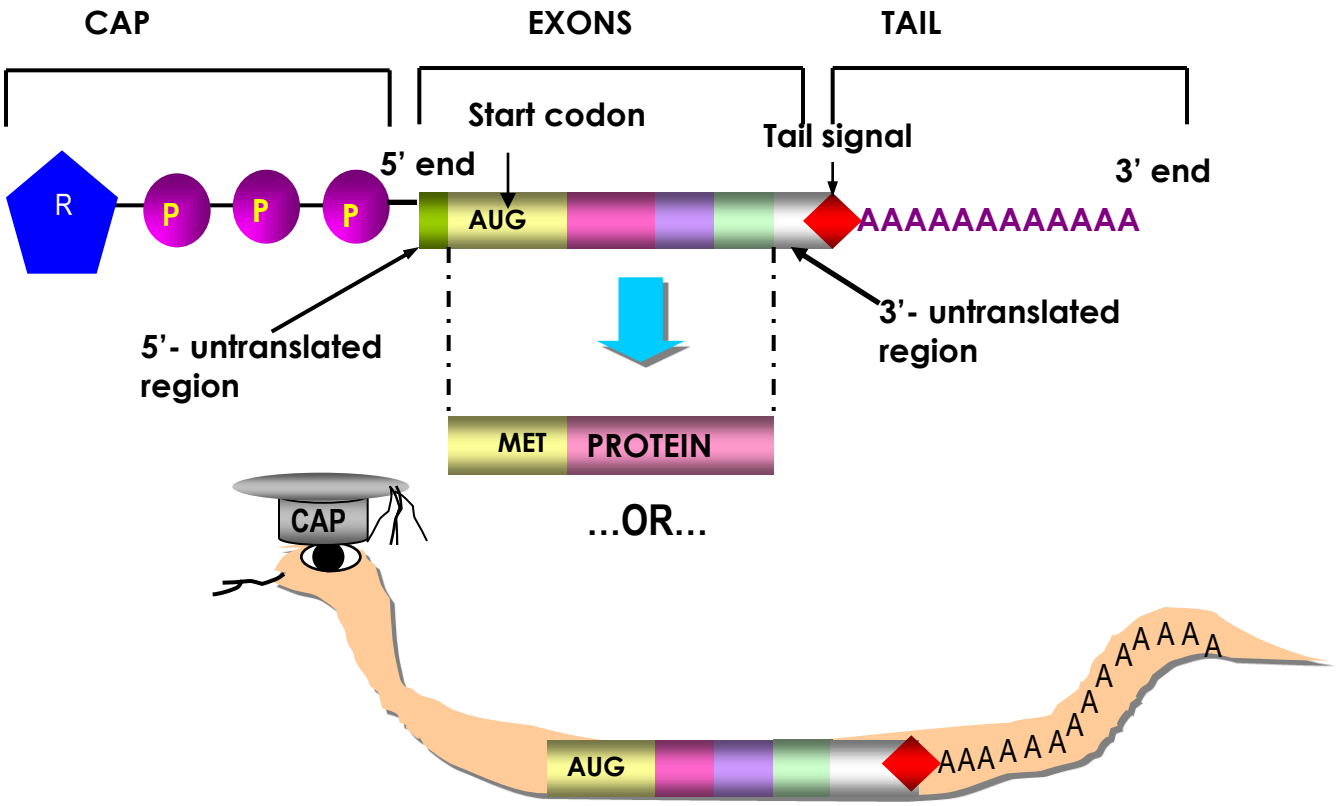
- Each nucleus has many pores that allow molecules in or out in a carefully controlled manner. **Each nuclear pore is guarded by a platoon of proteins**, but the details of just who is allowed in or out are still uncertain.

- We do know **once messenger RNA** has **received its cap and tail** and had its **introns spliced out**, it is free to exit the nucleus. The splicing factors (those tricky little SNURPS) that bind to the RNA, prevent it from leaving until splicing is finished.

Leaving the nucleus



Mature Eukaryotic mRNA



Protein Synthesis in Eukaryotes

- You will be relieved to know that protein synthesis in higher organisms is much the same as in bacteria, with few major differences.

- The **ribosomes** of eukaryotic cells are a **bit bigger** than those of prokaryotic cells, and **contain several more proteins**.

- Due to this, the ribosomal subunits of higher organisms are referred to as the **40S** (small) and **60S** (large) subunits. Together they form an **80S** ribosome.

Binding of the 40S and 80S Ribosome

- In eukaryotic cells, the ribosomes, the **sites of protein synthesis**, are in the **cytoplasm**.

- However, **manufacture and processing of mRNA** occurs **INSIDE the nucleus**. Consequently, the messenger RNA molecule must be released from the nucleus before it can be bound to a ribosome and translate protein.

- In prokaryotic cells the ribosome can bind to mRNA and may get started making protein before the mRNA has been finished itself. Obviously, this cannot occur in eukaryotic cells.

- A whole group of special proteins, the **initiation factors**, are needed to get the eukaryotic ribosome ready for action.

- The **first transfer RNA**, the **small** (40S) ribosomal subunit, the **mRNA plus cap binding protein**, and the **large** (60S) ribosomal subunit are all bound individually by different initiation factors.

- The initiation factors assemble the complete ribosome plus the tRNAs and mRNA in the correct order.

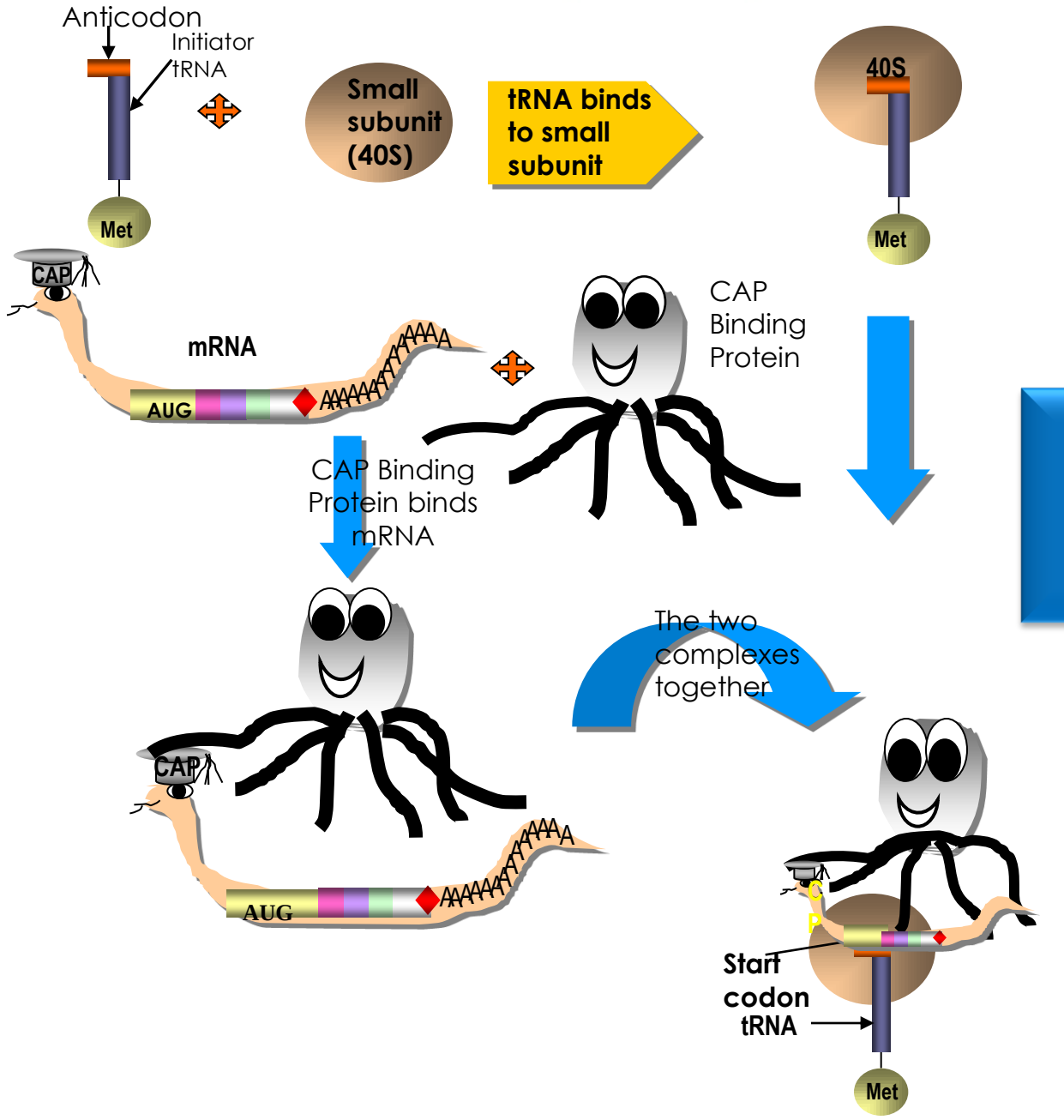
- First to act are the small (40S) ribosomal subunit and the **intiation tRNA**. Eukaryotic proteins are made starting with the amino acid **methionine**, just as in prokaryotes.

- There is a **special initiator tRNA** but, unlike prokaryotes, **no formyl-group** is used to label the first methionine (see fig. **Formation Of The 40s Eukaryotic Preinitiation Complex**).

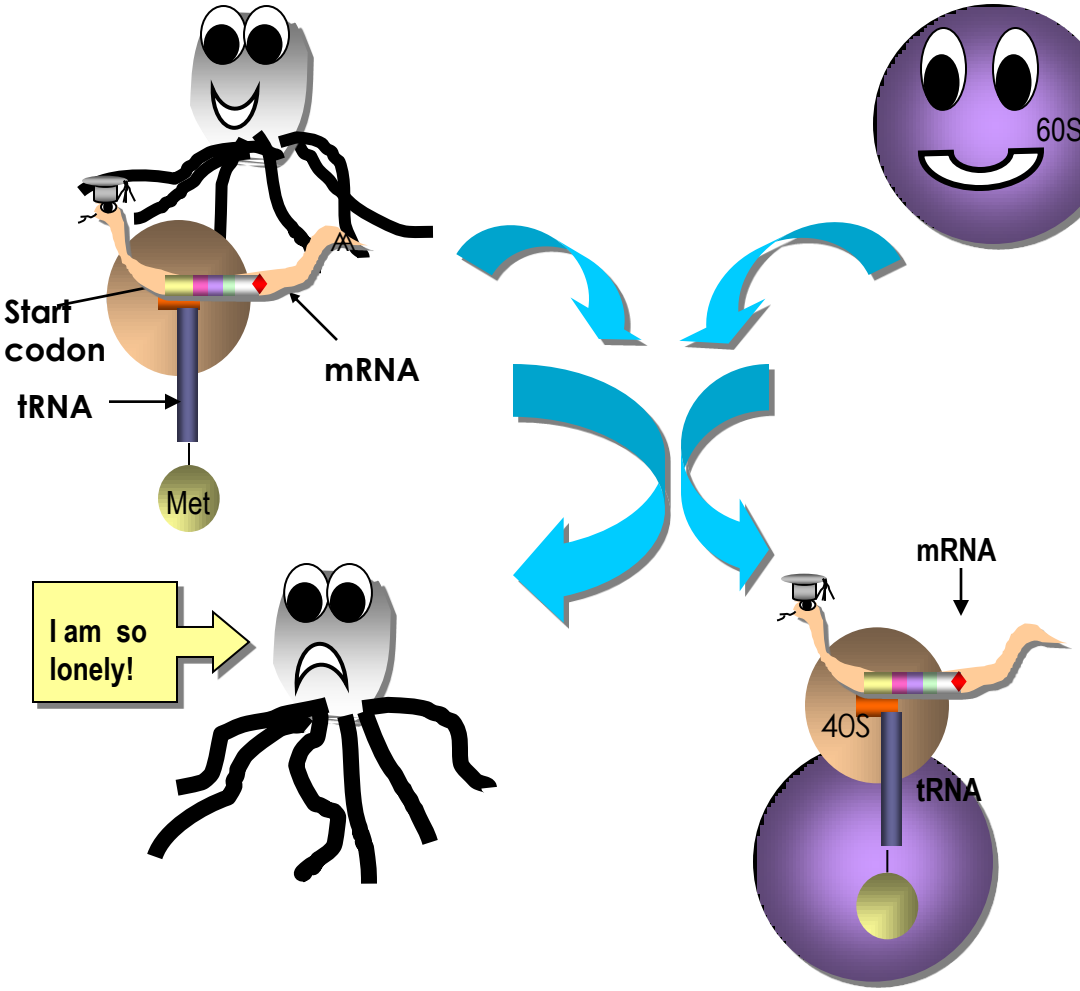
- Eukaryotic messenger RNA **does not have a ribosome binding site** (Shine-Dalgarno sequence) as in prokaryotes.

- Instead, it is recognized by a **special cap structure at the 5' end**. Cap binding protein binds the cap of mRNA and hand over the mRNA to the small ribosomal subunit.

- Because there is **no Shine-Dalgarno** sequence to align the mRNA in eukaryotes, the **AUG codon** of the mRNA is **recognized as the start site** for protein synthesis once the initiator tRNA has found the **start codon**, the large (60S) ribosomal subunit binds and protein synthesis starts (see **Formation Of 80s Initiation Complex**).



Formation Of The 40s Eukaryotic Preinitiation Complex



Formation of 80S Initiation Complex

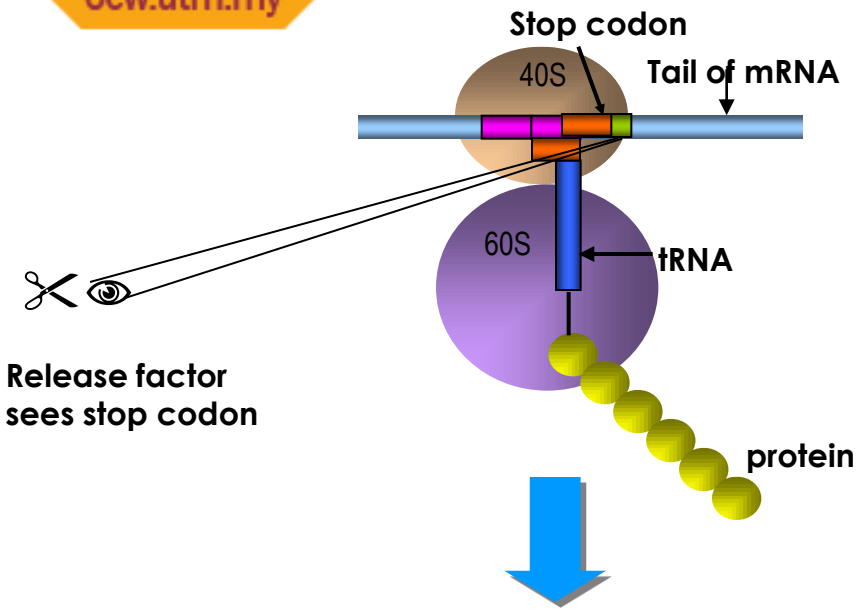
- The incoming amino acids are linked into a polypeptide chain, pretty much as in bacteria. **Eukaryotes** only have **a single coding sequence** on each messenger RNA.

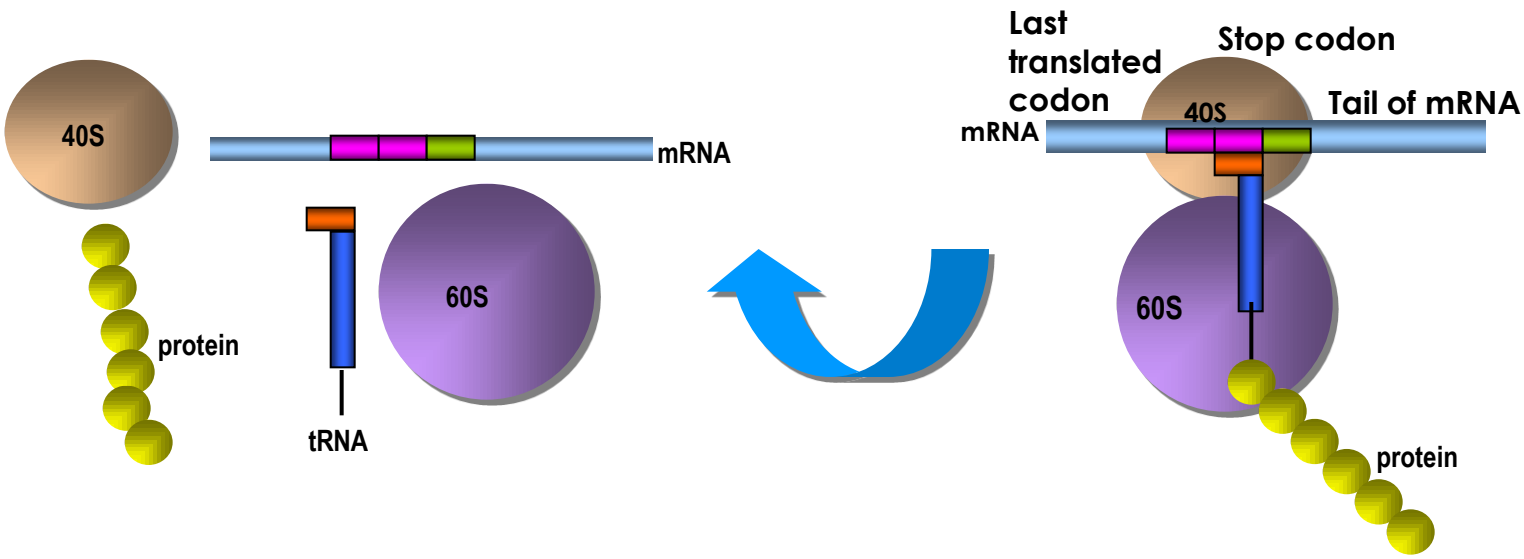
- Therefore, they make only a single protein per mRNA. Once the ribosome reaches the stop codon, it disassembles.

- The newly made protein, the mRNA, and the two ribosomal subunits are all released (see **Termination Of Protein Synthesis**).

- This is under the control of a single protein which recognizes the stop codon, eukaryotic **release factor**.

Termination of Protein Synthesis





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